(54) Title: HUMAN CD26 AND METHODS FOR USE

(57) Abstract

A polypeptide fragment of CD26 (or analogs thereof) capable of disrupting the naturally-occuring binding interaction between CD45 and CD26, and a method of screening such compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which method includes the steps of: a) providing a first and a second sample of cells expressing both CD26 and CD45; b) incubating the first sample in the presence of a candidate compoud; c) incubating the second sample in the absence of the candidate compound; d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD26 antibody; e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and f) determining whether the amount of CD45 present in the first immunoprecipitate is less than the amount of CD45 present in the second immunoprecipitate, the presence of a lesser amount of CD45 in the first immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the binding.

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HUMAN CD26 AND METHODS FOR USE

Background of the Invention

The field of the invention is human T cell activation antigens.

CD26 is a human T cell activation antigen originally identified by its reactivity with the
5 monoclonal antibody Ta1 (Fox et al., *J. Immunol.* 133:1250, 1984). CD26 has recently been shown to be identical to human dipeptidyl peptidase IV (EC 3.4.14.5) (Ulmer et al., *Scand. J. Immunol.* 31:429, 1990; Barton et al., *J. Leukocyte Biol.* 48:291, 1990). Dipeptidyl
10 peptidase IV (DPPIV) is a serine exopeptidase which is capable of cleaving x-proline or x-alanine (where x is any amino acid) from the amino terminus of certain peptides.

CD26 is recognized by a second monoclonal
15 antibody, anti-1F7 (Morimoto et al., *J. Immunol.* 143:3430, 1989). Dang et al. (*J. Immunol.* 144:4092, 1990) report that solid phase-immobilized anti-1F7 mAb is capable of inducing proliferation of human CD4^{+} T lymphocytes in conjunction with submitogenic doses of
20 anti-CD3 or anti-CD2 antibodies. They suggest that the CD26 antigen is involved in CD3- and CD2-induced human CD4^{+} T cell activation.

Summary of the Invention

In one aspect, the invention features a
25 polypeptide fragment of CD26 having an amino acid sequence substantially identical to the amino acid sequence of CD26, except that amino acid residues 3-9 of the latter sequence have been deleted (Δ3-9, SEQ ID NO: 2). In preferred embodiments, the polypeptide has an

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amino acid sequence identical to the amino acid sequence
of SEQ ID NO: 2; the polypeptide is soluble under
physiological conditions; and the polypeptide is
substantially pure. Also within the invention is the
5 product of signal peptidase proteolytic cleavage of this
polypeptide, which would be a form of CD26 lacking
residues 1-34, 1-35, 1-36, or 1-37.

In a related aspect, the invention features a
nucleic acid encoding a polypeptide fragment of CD26
10 having an amino acid sequence substantially identical to
the amino acid sequence of Δ3-9 (SEQ ID NO: 2). In
another related aspect, the invention features a plasmid
which includes this nucleic acid, and preferably also an
expression control sequence.

15   In another aspect, the invention features a
polypeptide fragment of CD26 having an amino acid
sequence substantially identical to the amino acid
sequence of CD26 except that residues 24-34 of the latter
sequence are deleted (Δ24-34, SEQ ID NO: 3). In
20 preferred embodiments, the polypeptide has an amino acid
sequence identical to the amino acid sequence of SEQ ID
NO: 3; the polypeptide is soluble under physiological
conditions; and the polypeptide is substantially pure.
Also within the invention is the product of signal
25 peptidase proteolytic cleavage of this polypeptide, which
would be a form of CD26 lacking residues 1-34, 1-35, 1-
36, or 1-37.

In a related aspect, the invention features a
nucleic acid encoding a polypeptide fragment of CD26
30 having an amino acid sequence substantially identical to
the amino acid sequence of SEQ ID NO: 3 (Δ24-34). In
another related aspect, the invention features a plasmid
which includes the nucleic acid, and preferably also an
expression control sequence.

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Polypeptide fragments of CD26 which are soluble under physiological conditions generally lack most or all of the hydrophobic amino acid residues found near the amino terminus of the polypeptide depicted in SEQ ID NO:

5 1. This can be accomplished by genetically manipulating a nucleic acid encoding CD26 to delete the hydrophobic residues, or to delete enough of the N-terminal amino acids (e.g., residues 3-9 or 24-34) to leave the resulting polypeptide susceptible to cleavage by signal

10 peptidase. Other fragments of CD26 which are within the invention include those in which all or part of the putative dipeptidyl aminopeptidase catalytic site (Gly_{627} to Gly_{631}) is deleted. Such fragments, which include inter alia the deletion mutant shown in Fig. 15 (SEQ ID

15 NO: 11); fragments having additional deletions such as those in $\Delta 3$-9 (SEQ ID NO: 2) and $\Delta 24$-34 (SEQ ID NO: 3); and those missing the entire signal peptide region up to Ala_{35}, Thr_{36}, Ala_{37} or Asp_{38}, would constitute enzymatically inactive fragments of CD26 useful in the

20 screening assays of the invention, as well as for inhibiting complex formation between CD26 and/or CD45 and p43.

By "substantially pure" is meant a polypeptide or protein which has been separated from biological

25 macromolecules, (e.g., other proteins, carbohydrates, etc.) with which it naturally occurs. Typically, a protein or polypeptide of interest is substantially pure when less than 25% (preferably less than 15%) of the dry weight of the sample consists of such other

30 macromolecules.

By "physiological conditions" is meant an aqueous solution, whether in vivo or in vitro, having a pH and salt concentration similar to that found in serum. Phosphate buffered saline is an example of a commonly

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used buffer in which a polypeptide that is soluble under physiological conditions would be soluble.

By "substantially identical to CD26" is meant that at least 80%, preferably at least 90%, more preferably at
5 least 95%, most preferably at least 99%, of the amino acid sequence is identical to that of the corresponding portion of CD26, and any non-identical amino acids in the sequence are amino acid substitutions, preferably conservative, which do not eliminate the biological
10 activity of the molecule.

By "plasmid" is meant an extrachromosomal DNA molecule which includes sequences that permit replication within a particular host cell.

By "expression control sequence" is meant a
15 nucleotide sequence which includes recognition sequences for factors that control expression of a protein coding sequence to which it is operably linked. Accordingly, an expression control sequence generally includes sequences for controlling both transcription and translation: for
20 example, promoters, ribosome binding sites, repressor binding sites, and activator binding sites.

In another aspect, the invention features a polypeptide fragment of CD26 (or analogs thereof) capable of disrupting the naturally-occurring binding interaction
25 between CD45 and CD26. The term "analogs" refers to polypeptide fragments of CD26 having conservative and/or non-conservative substitutions for some of the amino acids of naturally-occurring CD26, having D-amino acids in place of some or all of the corresponding L-amino
30 acids, or having non-peptide bonds in place of some of the peptide bonds of CD26. Techniques for producing such analogs are well known in the art, and can be readily accomplished by those of ordinary skill. Preferably at least 85%, more preferably at least 95%, and most
35 preferably at least 99%, of the amino acids in the analog

are identical to the corresponding ones in CD26. It is important that the substitutions do not eliminate the ability of the polypeptide fragment to interfere with the naturally occurring association between CD26 and CD45.

5 In some instances, the removal of peptide bonds from a polypeptide compound is a desirable goal because the presence of such bonds may leave the compound susceptible to attack by proteolytic enzymes. Additionally, such peptide bonds may affect the biological availability of

10 the resulting therapeutic molecules. The removal of peptide bonds is part of a process referred to as "depeptidization". Depeptidization entails such modifications as replacement of the peptide bond (-CONH-) between two given amino acids with a spatially similar

15 group such as $-CH_2CH_2-$, $-CH_2-O-$, $-CH=CH-$ or $-CH_2S-$, generally by incorporating a non-peptide mimetic of the dipeptide into the chemically synthesized analog of the invention.

Polypeptides and analogs which disrupt the
20 interaction between CD26 and CD45 can be identified using the immunoprecipitation assay described herein below.

In another aspect, the invention features a method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which
25 method includes the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating the first sample in the presence of a candidate compound;

30 (c) incubating the second sample in the absence of the candidate compound;

(d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD26 antibody;

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(e) generating a second immunoprecipitate by adding to the second sample a second aliquot of the antibody; and

5   (f) determining whether the amount of CD45 present in the first immunoprecipitate is less than the amount of CD45 present in the second immunoprecipitate, the presence of a lesser amount of CD45 in the first immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the
10 binding.

As used herein, an anti-CD26 antibody is one capable of forming a specific immune complex with CD26, i.e., the antibody binds directly to CD26 but does not substantially bind directly to other molecules in the
15 assay of the invention.

In another aspect, the invention features a method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, which method includes the steps of:
20   (a) providing a first and a second sample of cells expressing both CD26 and CD45;

(b) incubating the first sample in the presence of a candidate compound;

(c) incubating the second sample in the absence of
25 the candidate compound;

(d) generating a first immunoprecipitate by adding to the first sample a first aliquot of an anti-CD45 antibody;

(e) generating a second immunoprecipitate by
30 adding to the second sample a second aliquot of the antibody; and

(f) determining whether the amount of CD26 present in the first immunoprecipitate is less than the amount of CD26 present in the second immunoprecipitate, the
35 presence of a lesser amount of CD26 in the first

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immunoprecipitate than in the second immunoprecipitate indicating that the candidate compound inhibits the binding.

In another aspect, the invention features a
5 monoclonal antibody which, when contacted under physiological conditions with a cell (preferably a eukaryotic cell such as a mammalian cell) expressing CD26 and CD45, interferes with the association of CD26 and CD45; and a method for assaying for such an antibody.
10 In yet another aspect, the invention features a method which includes:

(a) providing a cell which expresses CD45 on its surface; and

(b) introducing into the cell a nucleic acid
15 encoding CD26, such that the cell expresses CD26 on its surface.

In yet another aspect, the invention features a method which includes:

(a) providing a cell which expresses CD26 on its
20 surface; and

(b) introducing into the cell a nucleic acid encoding CD45, such that the cell expresses CD45 on its surface.

In other aspects, the invention includes a cell
25 transfected with a nucleic acid encoding CD26, the cell expressing both CD26 and CD45 on its surface; and a cell transfected with a nucleic acid encoding CD45, the cell expressing both CD26 and CD45 on its surface. In preferred embodiments, the cells are T-cells such as
30 Jurkat cells.

In another aspect, the invention features a method which includes:

(a) providing a cell which expresses neither CD26 nor CD45 on its surface; and

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(b) transfecting the cell with a nucleic acid encoding CD26 and a nucleic acid encoding CD45.

In yet another aspect, the invention includes a method of generating a hybridoma cell, which method

5   includes:

(a) providing a cell transfected with nucleic acid encoding CD26, such that the cell expresses CD26 on its surface;

(b) using the cell as an antigen to induce an

10 immune response in a subject animal; and

(c) fusing a B lymphocyte from the subject animal with a cell from an immortal cell line (i.e., a line of cells which can be maintained indefinitely in culture) to produce a hybridoma cell.

15   In a related aspect, the invention features a hybridoma cell generated by:

(a) providing a cell transfected with nucleic acid encoding CD26, such that the cell expresses CD26 on its surface;

20   (b) using the cell as an antigen to induce an immune response in a subject animal; and

(c) fusing a B lymphocyte from the subject animal with a cell from an immortal cell line to produce a hybridoma cell, wherein the hybridoma cell produces a

25 monoclonal antibody specific for CD26. Applicable methods of inducing an immune response in an animal by using cells as the antigen, and fusing B lymphocytes with immortal cells to produce hybridoma cells, are well known to those of ordinary skill in the art of making

30 hybridomas. The resulting hybridomas are then cloned and screened for production of monoclonal antibodies which bind to cells expressing the CD26 antigen, but not to identical cells which do not express the CD26 antigen.

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Also within the invention are cell-free preparations of CD26, or a fragment thereof, complexed with CD45, or a fragment thereof. Such complexes may be conveniently prepared by recombinant expression of each

5 of the relevant polypeptides in a manner that prevents their being anchored to the cellular membrane (e.g., by use of a soluble fragment of each), or by isolation of the full-length proteins from a cell membrane preparation, and by combining the two polypeptides to

10 form the desired complex either before or after removal of contaminating cellular constituents. Such complexes would be useful, e.g., for generating monoclonal antibodies specific for the complex, and for screening for compounds capable of interfering with the association

15 of CD26 and CD45.

Also within the invention are purified preparations of p43, a 43 kDa molecule which, like CD45, associates with CD26 in cells and therefore is thought to play a role in T cell activation, and cell-free

20 preparations of CD26 (or a fragment thereof) complexed with p43 (or a fragment thereof). The screening assay described above for compounds capable of inhibiting the interaction of CD26 and CD45 can be readily adapted to detect compounds (including fragments of CD26 or p43)

25 capable of inhibiting the interaction of CD26 and p43.

CD26 is known to play a role in T cell activation. By interfering with the normal functioning of CD26, one can control the process of T cell activation, and thus prevent such unwanted immune responses as transplant

30 rejection and certain autoimmune diseases. The information disclosed herein concerning proteins with which CD26 associates on the T cell provides the means for designing and screening compounds that interfere with CD26 function in the cell.

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Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

Detailed Description

5   The drawings are first briefly described.

Drawings

Fig. 1 depicts the nucletide sequence and deduced amino acid sequence (SEQ ID NO:1) of the cDNA clone for human CD26.

10   Fig. 2 depicts the results of an indirect fluoresence staining assay.

Fig. 3 is a pair of photographs of gels illustrating the results of immunoprecipitation analysis (panel A) and enzymatic activity analysis (panel B).

15   Fig. 4 is a set of graphs depicting the results of a $[Ca^{2+}]_i$ mobilization assay.

Fig. 5 is a graph illustrating the effect of various treatments on interleukin-2 production.

Fig. 6 is a photograph of a gel illustrating the 20 results of immunoblotting analysis.

Fig. 7 depicts the results of FACS analysis.

Figs. 8-12 are photographs of gels illustrating the results of immunoprecipitation assays.

Fig. 13 is a representation of the amino acid 25 sequence of CD26 in which the deleted amino acids of Δ3-9 (SEQ ID NO: 2) are indicated by a box, and the probable proteolytic cleavage sites of the signal peptidase are indicated by arrows.

Fig. 14 is a representation of the amino acid 30 sequence of CD26 in which the deleted amino acids of Δ24-34 (SEQ ID NO: 3) are indicated by a box, and the probable proteolytic cleavage sites of the signal peptidase are indicated by arrows.

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Fig. 15 depicts the amino acid sequence of a CD26 fragment lacking a portion of the carboxy terminal region of CD26 (SEQ ID NO: 11).

Sequencing and Characterization of CD26

5   Described below is the cloning and sequencing of a full-length CD26 cDNA. Also described are a series of experiments which demonstrate that: (1) modulation of CD26 from the surface of T lymphocytes leads to enhanced CD3ξ phosphorylation and increased CD4-associated p56^{lck}

10 tyrosine kinase activity; (2) CD26 is comodulated with CD45; and (3) CD26 and CD45 are closely associated.

Cells and Antibodies

        Human peripheral blood mononuclear cells (PBMC), E rosette-positive cells and PHA-activated T cells for

15 use in the experiments described below were prepared as follows.   Human PBMC were isolated from healthy volunteer donors by Ficoll-Hypaque density gradient centrifugation (LKB Biotechnology, Inc., Piscataway, NJ). Unfractionated mononuclear cells were separated into E

20 rosette-positive (E+) and E rosette-negative (E-) populations, and the E+ cells were depleted of contaminating monocytes as described (Morimoto et al., J. Immunol. 134:3762, 1985; Morimoto et al., J. Immunol. 134:1508, 1985; Matsuyama et al., J. Exp. Med. 170:1133,

25 1989). These T cells were used for experiments involving T cells in this report. E+ cells were stimulated with PHA (0.25 μg/ml) and rIL-2 (40 U/ml) for 7 days in RPMI 1640 medium supplemented with 10% human AB serum, 4mM L-glutamine, 25 mM HEPES buffer, 0.5% sodium bicarbonate,

30 and 1% penicillin/streptomycin (culture medium) and used as PHA blasts. The monoclonal antibodies used were anti-CD26 (Ta1/4EL-1C7, IgG$_1$; 1F7, IgG$_1$; 5F8, IgG$_1$), and anti-CD3 (T3/RW24B6; IgG$_{2b}$) (Fox et al., J. Immunol. 133:1250, 1984; Morimoto et al., J. Immunol. 143:3430, 1989;

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Morimoto et al., *J. Immunol.* 134:3762, 1985). Anti-CD29
(4B4; IgG$_1$) (Morimoto et al., *J. Immunol.* 134:3762, 1985)
was used as an isotype-matched control throughout.

Isolation of a CD26 cDNA

5   To isolate a CD26 cDNA, a cDNA library was
constructed from mRNA isolated from human PHA-activated T
cells using the CDM7 vector. Briefly, poly(A)+ RNA was
prepared from 4-day-old PHA-activated T cells by the
guanidinium isothiocyanate method (Chirgwin et al.,
10 *Biochem.* 18:5294, 1979), and an expression library was
prepared as previously described, except that the vector
CDM7, a precursor to CDM8 lacking polyoma sequences, was
employed (Aruffo et al., *Proc. Natl. Acad. Sci. USA*
84:8573, 1987; Seral et al., *Proc. Natl. Acad. Sci. USA*
15 87:3365, 1987). Recombinant hybrid plasmids were
transfected into COS cells, and CD26 expressing cells
were immunoselected with the monoclonal antibody, anti-
Ta1 (Aruffo et al., *supra*; Seed et al., *supra*). Reactive
cells were retained on antibody coated dishes, and
20 plasmids were recovered from transfected cells. Plasmid
DNAs were further selected by three additional rounds of
transfection and immunoselection. Two of eight clones
thus isolated were found to encode anti-Ta1 reactive
determinants. The two clones were identical by
25 restriction enzyme fragment mapping.
       Sequencing of both strands of the isolated 2.9 kb
CD26 cDNA by the dideoxy sequencing method revealed a
2298 base pair open reading frame beginning with an ATG
at nucleotide 11 which conforms to consensus
30 translational initiation sites (Fig. 1). The deduced
CD26 structure is a 766 amino acid residue polypeptide
with a molecular weight of approximately 88,300 (SEQ ID
NO: 1).

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Predicted Structure of CD26

The predicted CD26 polypeptide has a single stretch of hydrophobic amino acids in the N-terminal region between residues 7 and 28 (Fig. 1, boxed), which
5   is sufficiently long and hydrophobic to span a lipid bilayer (Davis et al., Cell 41:607, 1985). The sequence is preceded by six N-terminal residues which contain polar and charged residues, and is followed by charged residues that would not allow cleavage by signal
10 peptidase (von Heijne, Nucl. Acids Res. 14:4683, 1986). This sequence thus has the characteristics of a signal sequence of a type II membrane protein, which serves both to direct the translocation of the nascent protein across the membrane of the rough endoplasmic reticulum, and to
15 anchor the mature protein in the membrane (Hong et al., supra, 1990; Shipp et al., Proc. Natl. Acad. Sci. USA 85:4819, 1988; Thomas et al., J. Clin. Invest. 83:1299, 1989). Furthermore, the fact that potential N-glycosylation sites are located in the carboxy side of
20 the hydrophobic core (Fig. 1, short underlines) suggests that CD26 is a type II membrane protein. Therefore, the N-terminal 6 amino acid residues are predicted to be cytoplasmic, and the next 22 amino acids, which are primarily hydrophobic, are predicted to transverse the
25 cytoplasmic membrane. The 738 C-terminal amino acids constitute the predicted extracellular domain of CD26.

The predicted extracellular domain of CD26 may be conveniently divided into three regions: an N-terminal glycosylated region (residues 29 to 323), a relatively
30 cysteine-rich middle section (residues 324 to 551), and a C-terminal region (residues 552 to 766) (Fig. 1). The N-terminal region contains 8 of the 10 potential attachment sites for N-linked glycans (Fig. 1, short underlines) (Marshall, Ann. Rev. Biochem. 41:673, 1972), and one of
35 the 12 cysteine residues (Fig. 1, asterisks). In

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contrast, the subsequent cysteine-rich section contains 9
cysteines but only one N-linked glycosylation site. The
C-terminal region contains two cysteines, one N-linked
glycosylation site and a potential catalytic site (Fig.

5   1, double underline), the sequence G-W-S-Y-G at position
627 to 631. This sequence fits the consensus G-X-S-X-G
found in the active sites of serine proteases and
esterases, although tryptophan and tyrosine flanking the
catalytic serine are unusual residues at these positions

10 (Brenner, *Nature* 334:528, 1988).

Homology with the Other Proteins.

The predicted amino acid sequence of the human
CD26 antigen (SEQ ID NO: 1) is 85% homologous to the
deduced rat DPPIV enzyme sequence predicted from cDNAs

15 isolated from rat liver and kidney libraries.
Considering this high degree of homology and the fact
that anti-Ta1 antibody reacts with human liver and kidney
epithelium (Mobius et al., *Exp. Immunol.* 74:431, 1988),
the DPPIV enzyme present in those tissues is probably the

20 functional counterpart of the CD26 antigen. This high
degree of homology also supports the prediction of the
membrane topology of CD26, because rat DPPIV has been
shown to be a type II membrane protein (Hong et al.,
supra 1990).

25   Aside from the signal sequence, the greatest
homology between rat (Ogata et al., *supra*) and human
CD26/DPPIV proteins is in the C-terminal region, which
includes the putative catalytic site. In fact, the
sequences are identical from residues 624 to 724, and 94%

30 homologous from residues 552 to 766. This C-terminal
region is 46% homologous to a region of the predicted
yeast aminopeptidase B (DPAPB) sequence (Roberts et al.,
J. Cell. Biol. 108:1363, 1989). Further, CD26 amino acid
residues 107 to 233 are 36% homologous to DPAPB. The

35 yeast DPAPB enzyme is also a type II membrane dipeptidyl

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aminopeptidase, and is involved in the maturation of the
yeast pheromone alpha factor. The putative catalytic
sequence G-W-S-Y-G is conserved between human and rat
CD26/DPPIV and yeast DPAPB.

5   Recently the structures for CD10 and CD13 were
determined by cDNA cloning (Shipp et al., *supra*, Thomas
et al., *supra*). These antigens are ectoenzymes which
have neutral endopeptidase [EC. 3.4.24.11] and
aminopeptidase N [EC. 3.4.11.2] activities, respectively.
10 Although CD10 and CD13 are also type II membrane
proteins, there is no significant sequence homology
between these enzymes and CD26.

        Although the CD26 antigen is known to be a
functional collagen receptor (Dang et al., *J. Exp. Med.*
15 172:649, 1990), a homology search did not find
significant homology with any other known collagen-
binding proteins such as fibronectin, CD11b and the
integrins.

Characterization of CD26 Antigen expressed on Transfected
20 Jurkat Cells

        To characterize the cDNA-encoded CD26 antigen, the
human T cell leukemia line, Jurkat, was transfected with
the expression plasmid pSRα26, in which the CD26 cDNA was
placed under the control of the SRα promoter. Briefly,
25 the CD26 cDNA insert was cloned into the *PstI* and *EcoRI*
sites of the plasmid pCDLSRα296 (Takebe et al., *Mol.
Cell. Biol.* 8:466, 1988) by blunt-end ligation to create
the CD26 expression plasmid, pSRα-26. pSRα-26, digested
with *SalI*, and pSV2neo-SP (confers neomycin resistance to
30 host cells; Streuli et al., *EMBO J.* 8:787, 1989),
digested with *PvuI*, were used to co-transfect Jurkat
cells according to Streuli et al. (*supra*). Transfectants
were initially selected in RPMI1640 supplemented with 10%
fetal calf serum, 4mM glutamine and 1.0 mg/ml Geneticin
35 (Gibco/BRL, Bethesda, MD). Subsequently, the

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concentration of Geneticin was gradually decreased to 0.25 mg/ml during the selection period. Geneticin-resistant clones were further screened for CD3 and CD26 antigen expression by cell-surface staining as described

5 below. Transfectants were maintained in the above medium containing 0.25 mg/ml Geneticin.

Staining of cell surface antigens with monoclonal antibodies and flow cytometry analyses using an EPICS V cell sorter (Coulter) were performed as described by Dang

10 et al. (J. Immunol. 144:4092, 1990).

Parental Jurkat cells do not express detectable amounts of the CD26 antigen as determined by cell surface staining (Fig. 2), or by a binding assay with radiolabeled Ta1 monoclonal antibody. Northern blotting

15 analysis revealed that this cell line also does not express CD26 mRNA even after phorbol 12-myristate 13-acetate (PMA) treatment, which is known to induce CD26 expression (Dang et al., J. Immunol. 145:3963, 1990). Referring to Fig. 2, the Jurkat-CD26 transfectant 26.C28

20 had high expression of the CD26 antigen. On the other hand, another Jurkat-CD26 clone, 26.24, expressed only moderate levels of the antigen. Both transfectants were reactive with three anti-CD26 monoclonal antibodies (Ta1, 1F7, and 5F8) which define three distinct CD26 antigen

25 epitopes.

To study whether the CD26 antigen expressed on Jurkat T cell lines had the same characteristics as that on peripheral blood lymphocytes, immunoprecipitation experiments were carried out.

30   Briefly, cell surface proteins were labelled with lactoperoxidase-catalyzed iodination as described by Morimoto et al., (J. Imunnol. 143:3430, 1989). Immunoprecipitations (employing an NP-40 lysis buffer) using 1F7 monoclonal antibody were performed as described

35 by Morimoto et al. (supra, 1989). Immunoprecipitated

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proteins were separated by 8% SDS-PAGE under reducing conditions.

Referring to Fig. 3 (panel A), 1F7 monoclonal antibody immunoprecipitated a 110 kDa protein from the
5 CD26 transfected Jurkat cells (lanes 2 and 3) as well as from PHA blasts (lane 4). There was no detectable 110 kDa band in nontransfected (lane 1) and vector-only transfected Jurkat cells. Control anti-4B4 monoclonal antibody immunoprecipitated a comparable amount of 130
10 kDa protein from each of the cell lines. Interestingly, 1F7 immunoprecipitated an additional 43 kDa protein from both transfectants and PHA blasts. Similar results were observed using peripheral blood T cells. This 43 kDa protein may contribute to T cell activation through its
15 association with CD26.

DPP-IV enzymatic activity was measured using an Enzyme Overlay Membrane system (EOM, Enzyme System Products, Dublin, CA). Briefly, lysates were incubated with SDS sample buffer for 1 hr at room temperature and
20 separated by SDS-PAGE under non-reducing conditions. Following electrophoresis, the EOM moistened with 0.5M Tris-HCl, pH 7.8, was placed on the surface of the gel and this sandwich was incubated for 20 min in a humidified box at 37°C. The reaction was monitored by
25 long wavelength ultraviolet light. Referring to Fig. 3, panel B, DPPIV enzymatic activity was associated with a 160 kDa protein in both transfectants (lanes 2 and 3) and PHA blasts (lane 4), but not in parental Jurkat cells (lane 1), or vector-only transfected cells. It should be
30 noted that the DPPIV enzyme activity was stable in both non-reducing and reducing conditions but disappeared after boiling of the samples. While the apparent molecular weight of CD26 was 160,000 for preparations that were not boiled prior to electrophoresis, the
35 molecular weight of CD26 antigen was 110,000 if the

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protein was boiled prior to SDS-PAGE analysis. Similar results have been reported for rat hepatocyte DPPIV (Walburg et al., *Exp. Cell. Res.* 158:509, 1985). Taken together, the above-described results indicate that the

5   CD26 antigen expressed on the transfected Jurkat cells was the same as that on peripheral blood T cells.

Functional Analysis of CD26 Antigen on Jurkat Transfectants

To determine whether the CD26 antigen expressed on

10 transfected Jurkat cells has biological activity similar to that of CD26 expressed on peripheral blood T cells, we examined $[Ca^{2+}]_i$ mobilization induced by CD26 antigen triggering.

Briefly, loading of indo-1 pentaacetoxymethyl

15 ester (Calbiochem, San Diego, CA) into cells and the measurement of its fluorescence by flow cytometry were performed as described by (Blue et al., *J. Immunol.* 140:376, 1988). Indo-1-loaded cells were preincubated for 1-2 minutes with antibodies and the basal

20 intracellular calcium levels were determined for 33 seconds before the addition of polyclonal goat anti-mouse antibody (10 μg/ml) (Tago, Burlingame, CA). The RW24B6 anti-CD3 antibody was titrated in this system to determine the submitogenic dose for triggering each cell

25 type. After preincubation of each transfectant with anti-CD26 and/or a submitogenic dose of anti-CD3, anti-mouse antibody was added (time point of addition indicated by small arrows in Fig. 4). Antibody concentrations were 1 μg/ml for anti-1F7 and 20 ng/ml for

30 anti-CD3.

Referring to Fig. 4, crosslinking of anti-CD26 and submitogenic doses of anti-CD3 with goat anti-mouse immunoglobulin on CD26 transfectants resulted in greater $[Ca^{2+}]_i$ mobilization than crosslinking of anti-CD3 alone.

35 These antibodies did not induce $[Ca^{2+}]_i$ mobilization

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without cross-linking. It is well known that the $[Ca^{2+}]_i$ mobilization signal is divided into two phases: the initial transient rise, and the sustained increase phase (Gardner, *Cell* 59:15, 1989; Goldsmith et al., *Science*

5   240:1029, 1988). For both CD26 transfectants, the anti-CD26 and anti-CD3 crosslinking induced a strong initial $[Ca^{2+}]_i$ increase (Fig. 4). In addition, for the clone 26.C28, crosslinking induced a sustained increase of the $[Ca^{2+}]_i$ level as well (Fig. 4). The differential pattern

10 of $[Ca^{2+}]_i$ mobilization of the two transfectants may be attributed to the difference in the amount of CD26 antigen expressed by these two transfectants. The enhanced $[Ca^{2+}]_i$ mobilization was specific because, as was reported for peripheral blood T cells (Dang et al., *J.*

15 *Immunol.* 145:3963, 1990), crosslinking of the CD26 antigen alone did not induce $[Ca^{2+}]_i$ mobilization. Furthermore, crosslinking of anti-CD26 and anti-CD3 did not enhance the $[Ca^{2+}]_i$ mobilization of nontransfected or vector-only transfected Jurkat cells, and crosslinking of

20 the isotype-matched control antibody, anti-4B4, did not result in enhanced $[Ca^{2+}]_i$ mobilization of the transfectants. Similar to the data observed with transfectants, a small but significant transient rise in $[Ca^{2+}]_i$ mobilization was observed in normal resting T

25 cells following CD26 and CD3 crosslinking.

IL-2 production by transfected cells cultured in antibody-coated plates was measured as described by Dang et al., *J. Immunol.* 144:4092, 1990), except that the cell concentration was adjusted to 2×10^6 cell/ml. After 24 hr

30 of culture, supernatants were assayed for IL-2 production using ELISA (R&D system, Minneapolis, MN). Referring to Fig. 5, incubation of the clone 26.C28 transfectants with solid-phase-immobilized anti-1F7 and anti-CD3, which mimicked the crosslinking by anti-mouse antibody, induced

35 the production of a significant amount of IL-2 (striped

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bar), as compared to the control, vector-only transfected, Jurkat cells (solid bar). These results indicate that the CD26 Jurkat transfectants were functionally similar to peripheral blood T cells.

5 Moreover, the above data indicate that the stimulatory effect of anti-CD26 and anti-CD3 crosslinking in T cells was in part mediated by an enhancement of $[Ca^{2+}]_i$ mobilization. Since it is well known that the transient rise, as well as the sustained increase, in $[Ca^{2+}]_i$ is

10 necessary for IL-2 production (Gardner, *supra*; Goldsmith, *supra*), the sustained increase of the $[Ca^{2+}]_i$ observed in clone 26.C28 may be the basis for enhanced IL-2 production seen with the transfectant following anti-CD26 and anti-CD3 stimulation. Thus, the data obtained using

15 Jurkat CD26 transfectants provide direct evidence that the CD26 antigen plays an integral role in T cell activation.

Co-association of CD26 and CD45

The experiments described below demonstrate that

20 modulation of CD26 on the surface of T lymphocytes by anti-CD26 monoclonal antibody leads to enhanced phosphorylation of CD3 and increased p56^{lck} tyrosine kinase activity. Modulation experiments described below demonstrate that CD26 is co-modulated with CD45.

25 Finally, immunoprecipitation assays described below demonstrate that CD26 and CD45 are closely associated. Taken together, the results indicate that an interaction between CD26 and CD45 increases p56^{lck} tyrosine kinase activity, CD3 chain phosphorylation, and T lymphocyte

30 activation.

Enhancement of CD3ζ Phosphorylation Following anti-CD26 (1F7) Treatment

To evaluate the effect of anti-CD26 antibodies on one of the earliest signaling events in T cell

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activation, we investigated their role in the tyrosine phosphorylation of CD3ζ.

Immunoblotting analysis of tyrosine phosphorylation of CD3ζ was performed as described by
5 Vivier et al. (*J. Immunol*. 146:206, 1990). Briefly, peripheral blood T cells (10×10^6 per sample) were incubated in culture media alone or with anti-CD26 (1F7; 1:100 ascites dilution) for various times at 37°C. Cells were then extensively washed in ice cold PBS containing
10 5mM EDTA, 10mM NaF, 10mM sodium pyrophosphate, and 0.4mM sodium vanadate, then solubilized in lysis buffer (1% NP-40, 150mM NaCl, 50mM Tris HCl, pH 8.0, 5mM EDTA, 1mM PMSF, 10mM iodoacetamide, 10mM NaF, 10mM sodium pyrophosphate, 0.4mM sodium vanadate) for 15 min on ice.
15 After removing insoluble material by centrifugation at 12,000 rpm for 15 min, samples were combined with an equal volume of sample buffer (2% SDS, 10% glycerol, 0.1M Tris [pH 6.8] 0.02% bromophenol blue), reduced with 5% 2-mercaptoethanol, and separated on 12% SDS-polyacrylamide
20 gels. After separation on SDS-PAGE, cell lysates were transferred to nitrocellulose, and developed using ^{125}I-labelled anti-phosphotyrosine (UBI, NY; 100,000 cpm/ml in PBS containing 1% BSA). Affinity-purified anti-phosphotyrosine was iodinated to a specific radioactivity
25 of 10-20 μCi/μg protein using iodobeads (Pierce Chemical Co., Rockford, IL).

Referring to Fig. 6, a 21 kD tyrosine phosphoprotein (p21), which has been previously identified in T cells stimulated with various stimuli as
30 phosphorylated CD3ζ (Vivier et al., *supra*, 1990; Vivier et al., *J. Immunol*. 146:1142, 1991; Ashwell et al., *Annu. Rev. Immunol*. 8:139, 1990), was detected at a constitutive level in samples not treated with anti-CD26 (lane 1). Anti-CD26 treatment significantly increased
35 the phosphorylation of CD3ζ over the constitutive level

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after 1 hour of anti-CD26 incubation (lane 2). The level
of phosphorylated CD3ζ gradually increased with time,
reaching a maximum level after 4 hours of anti-CD26
incubation (lanes 3 and 4; 2 and 4 hours of anti-CD26
5 treatment respectively), and gradually decreased upon
longer incubation (lanes 5 and 6; 6 and 8 hours of anti-
CD26 treatment respectively). The total amount of CD3ζ
chain (phosphorylated and non-phosphorylated) present,
determined by immunoblotting the same membrane with an
10 anti-CD3ζ mAb, was similar in all samples. Although
anti-CD26 by itself can not induce T cell proliferation,
these results show that CD26 modulation provides an
initial T cell activation signal as measured by enhanced
CD3ζ phosphorylation.

15 <u>Comodulation of CD26 and CD45 by anti-CD26 Antibody (1F7)</u>
<u>Treatment</u>

    The fact that the cytoplasmic domain of CD26
(DPPIV) in the rat includes only six amino acid residues
suggests that CD26 might be associated with another
20 molecule which acts in a signal transducing capacity, as
has been found in the case of the IL-6 receptor and the
IL-2 (p55) receptor (Taga et al., *Cell* 58:573, 1989; Robb
et al., *J. Exp. Med.* 165:1201; 1987). The experiments
described below indicate that CD26 is associated with
25 another cell surface molecule, CD45. Human peripheral
blood T cells were used in the experiments described
below and obtained as described by Dang et al. *J.
Immunol.* 144:4092, 1990. Anti-CD26 (1F7) induced
modulation was performed as previously described (by Dang
30 et al. *J. Immunol.* 145:3963, 1990). Briefly, peripheral
blood T cells were incubated overnight at 37°C in medium
containing anti-CD26 (1F7) at 1:100 ascites dilution.
Cells were then collected, washed and stained with anti-
CD26 (1F7) and FITC-conjugated goat anti-mouse IgG; or
35 they were stained with anti-CD45RA (2H4)-PE, anti-CD2-PE,

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anti-CD3-PE (Coulter) or biotinylated anti-CD45RO (UCHL-1) and PE-conjugated avidin.

Flow cytometry analysis was performed using an Epics V cell sorter (Coulter Electronics) as previously
5 described (Morimoto et al., J. Immunol. 143:3430, 1989). The negative control of each fluorescence was less than 5%. The FACS analysis presented in Fig. 7 are representative of three separate experiments. As shown in Fig. 7, overnight incubation with anti-CD26 led to a
10 significant reduction in CD26 expression on T cells. Interestingly, while CD26 modulation did not have any detectable effect on CD2, CD3 or CD45RA expression, the expression of CD45RO, particularly the high fluorescence peak of CD45RO, was markedly reduced. In addition,
15 modulation of CD2, CD3, or CD4 with respective antibodies had no effect on CD45RO expression. Thus, the co-modulation of CD45RO induced by anti-CD26 treatment appears to be specific for this structure.

Co-immunoprecipitation of CD26 with CD45

20 The immunoprecipitation experiments described below provide evidence of a direct association between CD26 and CD45. Peripheral blood T cells (50x10^6) were labeled at the surface by lactoperoxidase-catalyzed iodination and immunoprecipitated from NP-40 lysis buffer
25 (0.5% NP-40, 140mM NaCl, 1mM PMSF, 5mM EDTA, 50mM Tris HCl [pH 7.4]) or digitonin lysis buffer (1% digitonin, 0.12% Triton X-100, 150mM NaCl, 1mM PMSF, 20mM Triethanolamine [pH 7.8]) using anti-CD26 (Ta1, Coulter Immunology, Hialeah, FL; or 1F7, Dr. C. Morimoto, Dana-
30 Farber Cancer Institute, Boston, MA) and anti-CD45 (GAP 8.3, Berger et al., Human Immunol. 3:231, 1981) as previously described by Morimoto et al. (J. Immunol. 143:3430, 1989) and Anderson et al. (Nature 341:159, 1989). All samples were analyzed under reducing
35 conditions.

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For immunodepletion studies, peripheral blood T cells were labeled and lysed in digitonin lysis buffer as described above. The lysates were precleared by four successive immunoprecipitations with anti-CD45 (GAP 8.3,
5   American Type Culture Collection, Bethesda, MD) or anti-CD1 (T6) and then precipitated by anti-CD26 and anti-CD45.

Digestion with V8 protease from *S. aureus* was carried out during gel electrophoresis as described by
10 Cleveland et al. (*J. Biol. Chem.* 252:1102, 1977). After the first gel electrophoresis, gel slices containing the high molecular weight proteins co-precipitated with CD26 and CD45 proteins were excised and polymerized into the stacking gel of a 15% SDS-polyacrylamide gel. 2.5 μg of
15 V8 protease in 10 μl of sample buffer (0.1% SDS, 0.125M Tris-HCl [pH 6.8], 10% glycerol, 0.1% bromophenol blue) were added to wells above the polymerized gel slices. Gel electrophoresis was carried out uninterrupted for 12 hours.

20   Fig. 8 presents the results of immunoprecipitation analysis without prior depletion. Surface labeled T-lymphocytes were solubilized in NP-40 (lanes 1-4) or digitonin (lanes 5-8) and immunoprecipitated with anti-CD1 (T6) as a negative control (lanes 1 and 5); anti-CD26
25 (1F7, lanes 2 and 6); anti-CD26 (Ta1, lanes 3 and 7); or anti-CD45 (GAP 8.3, lanes 4 and 8).

While anti-CD26 (Ta1 and 1F7) antibodies precipitated a 110KD molecule from NP-40 lysates under reducing conditions, in digitonin lysates these same
30 antibodies precipitated two major proteins at 180 and 190kD and minor bands at 205 and 220kD in addition to the 110KD band. These additional bands display similar mobility to the CD45 control immunoprecipitates. In this regard, utilizing digitonin lysates or chemical cross-
35 linkers, others have found an association of CD45 with

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Thy-1, CD3, and CD2 (Volarevic et al., *Proc. Natl. Acad. Sci. USA* 87:7085, 1990; Schraven et al., *Nature* 345:71, 1990).

To provide further evidence that the high
5 molecular weight structure which co-precipitated with CD26 is CD45, we carried out both sequential immunodepletion and one-dimensional peptide mapping studies using V8 protease.

Fig. 9 presents the results of immunoprecipitation
10 analysis of samples previously depleted for CD45 using anti-CD45 antibody (GAP 8.3, lanes 4-6) or, as a control, CD-1 using anti-CD1 antibody (T6, lanes 1-3). After depletion, anti-CD26 (1F7, lanes 1 and 4), anti-CD26 (Ta1, lanes 2 and 5), or anti-CD45 (GAP 8.3, lanes 3 and
15 6) was used for immunoprecipitation. As can be seen in Fig. 9, depletion of CD45 resulted in a complete loss of the high molecular weight structures in the CD26 immunoprecipitate (lanes 4, 5). Furthermore, V8 protease-dependent digestion of the high molecular weight
20 molecules co-precipitated with either CD26 and CD45 yielded identical peptide patterns (Fig. 10). Although CD26 comodulated only with CD45RO (the 180kD isoform), the immunoprecipitation experiments suggest that CD26 is also associated with the 190kD isoform of CD45, and to a
25 lesser degree, with the 205 and 220kD isoforms as well. These observations are consistent with earlier studies demonstrating that CD26 was preferentially expressed on CD45RO+ helper T cells, which are known to preferentially express both the 180 and 190kD isoforms of CD45 (Morimoto
30 et al., *J. Immunol.* 143:3430, 1989; Rudd et al., *J. Exp. Med.* 166:1758, 1987; Terry et al., *Immunology* 64:331, 1988).

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Enhancement of the Kinase Activity of p56[lck] following anti-CD26 (1F7) Treatment

Recent studies have demonstrated that the cytoplasmic domain of CD45 has PTPase activity which
5 regulates T cell activation pathways through dephosphorylation of phosphotyrosine (Charboneau et al., *Proc. Natl. Acad. Sci. USA* 85:7182, 1988; Ledbetter et al., *Proc. Natl. Acad. Sci., USA* 85:8628; Pingel et al., *Cell* 58:1055, 1989; Koretzky et al., *Nature* 346:66,
10 1990). One of the potential substrates for the CD45 PTPase is the tyrosine kinase p56[lck] (Osergaard et al., *Proc. Natl. Acad. Sci. USA* 86:8959, 1989; Mustelin et al., *Proc. Natl. Acad. Sci. USA* 86:6302, 1989), which itself may be involved in the CD3 chain phosphorylation
15 (Veillette et al., *Nature* 338:257, 1989). CD26 may function in this system by enhancing CD3 phosphorylation through its association with CD45. If this model is correct, incubation with anti-CD26 (1F7) should alter p56[lck] kinase activity as measured by *in vitro*
20 autophosphorylation.

To analyze *in vitro* kinase activity, samples of 3.0×10^7 T lymphocytes were incubated in culture media with anti-CD26 (1F7) for various periods of time at 37°C. Immunoprecipitation and kinase analysis was then carried
25 out as described by Rudal et al. (*Proc. Natl. Acad. Sci. USA* 85:5190, 1988). Cells were then solubilized in lysis buffer (1% NP-40, 20 mM TRIS-HCl [pH 8.0], 150 mM NaCl, 0.4 mM sodium vanadate, 0.5 mM EDTA, 10 mM NaF, 10 mM sodium pyrophosphate, 1 mM PMSF) for 30 min at 4°C. CD4
30 was immunoprecipitated from lysates containing equivalent amounts of total protein (500 μg) by a combination of anti-CD4 (19thy5D7; IgG2) and protein A-Sepharose. The immunoprecipitates were then washed extensively with lysis buffer prior to incubation with 30 μl of 25 mM
35 Hepes containing 0.1% NP-40, and 10μCi of [λ-^{32}P]ATP (ICN,

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Costa Mesa, CA). After incubation of 15-30 min at 25°C, the reaction was stopped by the addition of sample buffer and the reaction products were resolved on 9% SDS-PAGE.

As shown in Fig. 11, the PTK activity of $p56^{lck}$

5   precipitated with CD4 significantly increased after 1 hour of incubation with anti-CD26 (lane 2) compared to a no-anti-CD26 control (lane 1). The kinase activity was even higher after 2, 3 or 4 hours of incubation with anti-CD26 (lanes 3-6, respectively). Concomitantly, the

10 expression of CD26 on T cells treated with anti-CD26 (1F7) began to decrease within 1 hour of incubation and continued to decline as previously described (Dang et al., *J. Immunol.* 145:3936, 1990). Similar results were obtained when another anti-CD26 (Ta1) antibody was used.

15 Nevertheless, incubation of cells with control anti-Class I MHC or anti-VLA 4 mAbs did not alter $p56^{lck}$ activity. The above results support the notion that the interaction of CD26 with CD45 enhances $p56^{lck}$ activity.

The kinetics of $p56^{lck}$ PTK activity (Fig. 11) and

20 tyrosine phosphorylation of CD3 (Fig. 6) showed a similar pattern. This similarity supports the conclusion that tyrosine phosphorylation of CD3 induced by anti-CD26 is related to the PTK activity of $p56^{lck}$. In addition, the similar kinetics also showed that the increase in $p56^{lck}$

25 PTK activity quickly affects the phosphorylation of CD3, as reported previously (Veillett et al., *supra*). While the peak of the $p56^{lck}$ PTK activity or phosphorylation of CD3 induced by various stimuli is observed within minutes (Vivier et al., *supra*; Veillette et al., *supra*), the peak

30 of either $p56^{lck}$ or CD3 phosphorylation induced by anti-CD26 treatment was observed after hours. In this regard, although the close relationship between CD45 PTPase activity and $p56^{lck}$ PTK activity has been reported (Ostergaarol et al., *supra*; Mustelin et al., *supra*;

35 Veillette et al., *supra*), the regulation of PTPase

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activity of CD45 has not yet been established.
Therefore, it is possible that the change in PTPase
activity or the interaction between CD45 PTPase and p56^{lck}
may require a relatively long time period following anti-
5 CD26 treatment.   It is also possible that the interaction
between CD45 PTPase and p56^{lck} is via an indirect rather
than a direct mechanism.

CD26 is broadly distributed on non-hematopoietic
cells.   However, since the expression of CD45 is largely
10 restricted to leukocytes, the association between CD26
and CD45 is probably found only on leukocytes.   On the
other hand, membrane-linked PTPases such as CD45 have
been found on non-hematopoietic cells (Streuli et al., *J.
Exp. Med.* 168:1553, 1988; Streuli et al., *Proc. Natl.*
15 *Acad. Sci. USA* 86:8698, 1989; Lau et al. *Biochem J.*
257:23, 1989).   Although the functional role of CD26 on
nonhematopoietic cells is unclear, it is possible that
CD26 is associated with the membrane-linked PTPase on
nonhematopoietic cells.

20   In summary, we have demonstrated that anti-CD26-
induced modulation resulted in enhanced CD3
phosphorylation and increased p56^{lck} PTK activity.   Both
observations are consistent with the enhanced
proliferative response of T cells following CD26
25 modulation.   These observations further suggest that the
physical association of CD26 with CD45 may be key for
CD26-mediated T cell signaling pathways.   CD26 is known
to be the membrane-associated ectoenzyme DPPIV which can
cleave N-terminal dipeptides from polypeptides with
30 either L-proline or L-alanine at the penultimate
position.   Although the natural ligand for CD26/DPPIV has
not yet been established, binding of the natural
substrate to the DPPIV enzyme may lead to cleavage and
alteration in the biologic activity of the ligand.   In
35 light of the close proximity of the CD26 and CD45

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molecules, it is possible that CD26 modulates the enzymatic activity of the CD45 PTPase or perhaps affects the accessibility of critical substrates. This process would then enhance T cell activation via the CD3 or CD2

5 pathway and could amplify the immune response *in vivo*. It should also be noted that increased numbers of CD26+ T lymphocytes have been found in both inflamed tissues and peripheral blood of patients with multiple sclerosis, Graves' Disease and rheumatoid arthritis (Hafler et al.,

10 *N. Engl. J. Med.* 312:1405, 1985; Nakao et al., *J. Rheumatol.* 16:904, 1989; Eguchi et al., *J. Immunol.* 142:4233, 1989), suggesting that these CD26+ T cells may play an important role in chronic inflammation and in subsequent tissue damage.

15 Soluble CD26 Fragments

Soluble fragments of CD26 are useful for interfering with CD26 activity. The fact that CD26 is a type II membrane protein suggests certain strategies for designing soluble fragments. For type II membrane

20 proteins, the signal sequence used to transfer the protein across a membrane also serves as an anchor to the membrane. The cleavage of the signal sequence after protein transfer which usually occurs for other secreted proteins does not occur in type II transmembrane

25 proteins. Thus, soluble forms of CD26 can be prepared by making its signal/anchor sequence accessible to a cellular proteolytic cleavage system. To accomplish this, the putative signal sequence of CD26 was shortened, as described below, since the 23 amino acid CD26 signal

30 sequence is longer than most natural occuring cleavable signal sequences (von Heijne et al., *J. Mol. Biol.* 184:99, 1985). This is expected to result in proteolytic cleavage of the expressed polypeptide at or near one of the residues Ala Thr Ala corresponding to positions 35-37

35 of wild type CD26, yielding a soluble fragment of CD26

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having at its amino terminus Ala$_{35}$, Thr$_{36}$, Ala$_{37}$ or Asp$_{38}$ of wild type CD26.

A first soluble CD26 construct is created by deleting the codons corresponding to amino acids 3-9 of
5 intact CD26 (shown as the boxed amino acids in Fig. 13). The amino terminal sequence of the expressed polypeptide is MKGLLG-- (SEQ ID NO: 4) rather than the original MKTPWKVLLGLLG-- (SEQ ID NO: 5), and the potential proteolytic cleavage sites are shown as arrows in Fig.
10 13. This deletion mutant is prepared by oligonucleotide directed mutagenesis (see below) using the following oligonucleotide:

5'-ACGCCGACGATGAAGGGACTGCTGGGTGCT-3' (SEQ ID NO: 6).

15   A second construct is generated by taking advantage of the following rules proposed for signal peptide cleavage: (1) the residue in position -1 must be small, i.e., either Ala, Ser, Gly, Thr, Cys, Gln; (2) the residue in position -3 must not be aromatic (Phe, His,
20 Tyr, Trp), charged (Asp, Glu, Lys, Arg), or large and polar (Asn, Gln); and (3) Pro must not be present at positions -3 through -1 (von Heijne, *Nuc. Acids Res.* 14:4683, 1986). Following these rules, we have designed a CD26 cDNA construct lacking codons corresponding to
25 amino acids 24 to 34 of wild type CD26 (illustrated as the boxed amino acids in Fig. 14). This deletion mutant encodes the amino acid sequence --IITVATADSR-- (SEQ ID NO: 7) instead of the original --IITVPVVLLNKGTDDATADSR-- (SEQ ID NO: 8), and the
30 potential proteolytic cleavage sites are shown as arrows in Fig. 14. This mutant is prepared by oligonucleotide-directed mutagenesis (see below) using the following oligonucleotide: 5'-ACCATCATCACCGTGGCTACAGCTGACAGT-3' (SEQ ID NO: 9).   Site-directed mutagenesis is
35 performed as follows. The 3.0 kb CD26 cDNA fragment

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generated by the *XbaI* treatment of the original plasmid CDM7-CD26 is inserted into the *XbaI* site of pTZ19u (Bio-rad). A recombinant plasmid which inserts the cDNA inverse to the *lacZ* gene on the plasmid is identified by restriction enzyme mapping and used for subsequent mutagenesis.

Using single-stranded DNA prepared from this plasmid as a template and the previously-described oligonucleotides as primers, oligonucleotide-directed mutagenesis is performed by the method of Kunkel (*Proc. Natl. Acad. Sci. USA* 82:488, 1985), using a commercially available kit (BioRad, Richmond, CA).

To obtain high level expression of soluble CD26, a new expression vector is constructed. First the *XbaI* CD26 cDNA fragment of pTZ19u-CD26 and the *HindIII-XbaI* vector fragment of Rc/CMV (Invitrogene, San Diego, CA) are treated with Klenow enzyme and ligated. The resulting plasmid is screened by restriction enzyme mapping for the insertion of the CD26 cDNA fragment under the control of the CMV promoter. This construct leaves one *XbaI* site just in front of the CD26 cDNA. Then, the *MluI-XbaI* CMV promoter DNA fragment of this plasmid DNA is exchanged with the *HindIII-XbaI* SRα promoter DNA fragment of pSRα-26 to give a final expression vector RcSRα-26. Next, the above mutant CD26 cDNAs are transferred to this expression vector. The *XbaI-DraIII* DNA fragment derived from the mutant cDNAs which encoded the mutant part and the wild type 2.0 kb *DraIII-HindIII* DNA fragment are ligated with the *XbaI-HindIII* vector fragment of RcSRα-26. The expression plasmid which has the Δ3-9 or Δ24-34 mutant CD26 cDNA is identified by restriction enzyme mapping and DNA sequencing. The resultant plasmids RcSRα-26.Δ3-9 and RcSRα-26.Δ24-34 are used to transfect Jurkat cells or CHO cells.

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Jurkat cells are transfected with these plasmids as described above except pSVneo-sp is omitted from the donor DNA mixture since the RcSRα plasmid already carries the neo resistance marker. Neo-resistant clones are
5   screened by metabolic labelling and immunoprecipitation (Harlow et al., eds. *Antibodies: a laboratory manual*, Cold Spring Harbor Laboratory, 1988) for the expression of soluble CD26. The transfectants which produce a large amount of soluble CD26 are used for protein production.
.10   CHO cells transfected with the DNA mixture of pMT2 and RcSRα-26.Δ3-9 or RcSRα-26.Δ24-34 are selected for their growing ability in α-medium and the production of soluble CD26. The expression of the soluble protein is amplified by culturing the transfected CHO cells in
15   medium containing an increasing amount of MTX. Although both Jurkat cells and CHO cells can provide the soluble form of CD26, the protein produced by Jurkat cells is preferred because of its human T cell origin.

Another approach to making fragments of CD26 is
20   illustrated by the following:

Ligation of the CD26 XbaI-SphI cDNA fragment to the vector RcSRα-26 XbaI-HindIII DNA fragment and the following synthetic DNA linker:

5'----------CATAGTAATCGATA
25   GTACGTATCATTAGCTATTCGA-------5' (SEQ ID NO: 10)
introduces an in-frame stop codon that results in deletion of the segment of CD26 from amino acid 594 to the carboxy terminus of the wild-type protein. This deletion mutant, which is shown in Fig. 15 (SEQ ID NO:
30   11), lacks the putative catalytic site of CD26 and has a new carboxy terminus of --GDKIMHA (SEQ ID NO: 12).

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CD26 Derivatives Capable of Disrupting CD26/CD45 Interaction

Other polypeptide fragments of CD26 can be produced by standard methods of protein synthetic
5 chemistry, using the information disclosed herein to design appropriate polypeptides and assay them for biological activity. A preferred method of producing such fragments, however, is by the use of recombinant DNA techniques. For example, the sequence of CD26 given in
10 Fig. 1 (SEQ ID NO:1) can be used to design oligonucleotides encoding fragments of CD26 containing deletions of nonessential CD26 amino acid residues from the beginning, the end, and/or any central portion of the protein; such oligonucleotides are chemically synthesized
15 by known methods and inserted into expression vectors for expression of a polypeptide fragment of CD26. Alternatively, one may manipulate the CD26 coding regions of CD26 expression plasmids by site-directed mutagenesis, as disclosed above for two such fragments of CD26, or by
20 insertion of a stop codon at an appropriate place in the coding sequence. The CD26 fragment can then be produced in transfected cultured cells in large quantities, purified by standard methods, and tested in an assay such as the immunoprecipitation assay described above, which
25 is useful for identifying fragments capable of disrupting the interaction of CD26 and CD45. Briefly, surface-labeled peripheral blood T cells which express both CD26 and CD45 (or any mammalian cells transfected with cDNAs encoding CD26 and CD45 so that both proteins are
30 functionally expressed on the cells' surfaces) are incubated in the presence and absence of a CD26 polypeptide fragment. The cells are lysed in digitonin lysis buffer, and anti-CD45 monoclonal antibody is used to immunoprecipitate CD45 and any proteins associated
35 with CD45. The amount of CD26 that co-precipitates with

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CD45 in the presence of a given polypeptide fragment can
be determined by known methods (e.g., by densitometer
readings of the labelled bands on an SDS-PAGE gel
analyzing the constituents of an immunoprecipitate) and
5   compared to the amount that co-precipitates with CD45 in
the absence of the polypeptide fragment. Alternatively,
one can instead use an anti-CD26 antibody and measure the
relative amounts of CD45 that co-precipitate with CD26 in
the presence and absence of the given polypeptide
10   fragment. If an anti-CD26 antibody is used, it is
preferred that the antibody does not substantially bind
to the competitor CD26 polypeptide; such binding
interferes with the assay. In either case, CD26
polypeptide fragments which interfere with the
15   interaction between CD26 and CD45 will decrease co-
precipitation.

An analysis similar to that described above can be
used to identify polypeptide fragments of CD45 which
disrupt CD26/CD45 interaction. When screening CD45
20   fragments, it is preferable to perform the
immunoprecipitation with anti-OCD26 antibody.

Association of p43 with CD26

When CD26 is immunoprecipitated from surface-
labelled T cells and the immunoprecipitate is analyzed on
25   SDS-PAGE, two bands are typically seen: one at 110kDa,
corresponding to CD26, and a second, much fainter band at
43kDa. This lower molecular weight protein is termed
"p43". Fig. 12 illustrates one such experiment, in which
E+ cells were labeled by lactoperoxidase-catalyzed
30   iodination and lysed in NP-40 lysis buffer for
immunoprecipitation as described above. Precipitates
were analyzed by 9% SDS-PAGE. Lane 1: anti-CD1 (T6) as
negative control; lane 2: anti-1F7; lane 3: anti-Ta1;
lane 4: anti-5F8 (another anti-CD26 monoclonal antibody);

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lane 5: anti-CD29 (4B4) as control. As shown in Fig. 12, anti-1F7 brought down an obvious 43kDa structure (lane 2) from surface-labeled T cells. On the other hand, this structure was detected faintly following anti-Ta1 or

5 anti-5F8 precipitation (lanes 3 and 4). This band was not detected following anti-CD1 or anti-CD29 precipitation (lanes 1 and 5). Similar results were seen when the cells were human thymocytes or from the human T cell lines H9 or Peer IV (data not shown). In other

10 anti-Ta1 or anti-5F8 immunoprecipitation experiments using T cells from other donors, the 43kDa band was sometimes more distinct than those shown in lanes 3 and 4 of Fig. 12. In addition, a third band at approximately 70 kDa is sometimes observed in these CD26

15 immunoprecipitation experiments. Because they are found in association with the 110 kDa CD26 molecule, both the 43 kDa molecule and the 70 kDa molecule may play important roles in T cell activation. Compounds (such as fragments or analogs of CD26) which interfere with the

20 association of CD26 with either p43 or the 70 kDa molecule may be detected by means of a screening assay patterned on those described above with respect to CD26 and CD45.

It is thought to be unlikely that anti-1F7 cross-

25 reacts with p43, since the density of the 43kDa band decreased after repeated preclearing by either anti-Ta1 or anti-5F8. Although the reasons for the variability in the detection of p43 are not clear, it is possible that the binding of anti-CD26 mAbs may generate conformational

30 changes in CD26, affecting the association of the 43 kDa molecule with the 110 kDa molecule. It is also possible that the Ta1 or 5F8 epitope may be close to the association site between the 43 and 110 kDa molecules, such that binding of these mAbs may inhibit the

35 ass ciation of these molecules with each other.

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P43 may be purified by affinity chromatography, using an anti-CD26 monoclonal antibody to purify the CD26-p43 complex from T cell membranes. P43 may then be separated from CD26 by SDS-PAGE, followed by HPLC if
5 further purification is necessary. Affinity chromatography with monoclonal antibodies, SDS-PAGE, and HPLC are all standard methods well known to those of ordinary skill in the art.

Hybridization probes based upon a partial amino
10 acid sequence of the purified protein may be used to select p43 cDNA from a T cell library. Alternatively, the partial amino acid sequence can be used to design PCR primers for priming synthesis of a partial p43 cDNA on mRNA templates, using standard methods, and the resulting
15 partial cDNA used as a probe to detect full-length p43 cDNA in a T cell library. This cDNA can be inserted in an expression plasmid and used to transfect cells which do not naturally express the p43 gene. Such cells would be useful for use as an antigen to develop anti-p43
20 monoclonal antibodies, and also as a means to study the role of p43 in T cell activation. They can also be used in the screening assay referred to above.

Northern Analysis Using a CD26 cDNA Probe

Analysis of the degree of expression of CD26 in
25 any given cell type or tissue type can be accomplished using the standard technique of Northern blotting, probing with a labelled, single stranded nucleic acid molecule derived from the coding region of CD26 cDNA. The probe would have a sequence based upon the sense
30 strand of SEQ ID NO: 1, which is complementary to CD26 mRNA, and preferably would be at least 8 nucleotides in length (more preferably at least 14 nucleotides, and most preferably at least 30). The probe may contain most or all of the entire coding sequence of CD26 cDNA. Such an
35 assay, which would be useful for diagnosing conditions

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characterized by the over- or under-expression of CD26 in a given cell type, such as T cells, would include the following steps:

      (a) providing a biological sample containing mRNA
5 of a cell;

      (b) contacting the sample with a single-stranded nucleic acid probe as described above; and

      (c) detecting hybridization of the probe with the sample, which hybridization would be indicative of the
10 presence of CD26 mRNA in the cell.

      Other embodiments are within the following claims.

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<u>SEQUENCE LISTING</u>

(1) GENERAL INFORMATION:

    (i) APPLICANT: Dana-Farber Cancer Institute, Inc.

    (ii) TITLE OF INVENTION: HUMAN CD26 AND METHODS FOR USE

    (iii) NUMBER OF SEQUENCES: 12

    (iv) CORRESPONDENCE ADDRESS:

        (A) ADDRESSEE: Fish & Richardson
        (B) STREET: 225 Franklin Street
        (C) CITY: Boston
        (D) STATE: Massachusetts
        (E) COUNTRY: U.S.A.
        (F) ZIP: 02110-2804

    (v) COMPUTER READABLE FORM:

        (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
        (B) COMPUTER: IBM PS/2 Model 50Z or 55SX
        (C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)
        (D) SOFTWARE: WordPerfect (Version 5.0)

    (vi) CURRENT APPLICATION DATA:

        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

    (vii) PRIOR APPLICATION DATA:

        (A) APPLICATION NUMBER: 07/832,211
        (B) FILING DATE: February 6, 1992

    (viii) ATTORNEY/AGENT INFORMATION:

        (A) NAME: Fraser, Janis K.
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        (C) REFERENCE/DOCKET NUMBER: 00530/055WO1

    (ix) TELECOMMUNICATION INFORMATION:

        (A) TELEPHONE: (617) 542-5070
        (B) TELEFAX: (617) 542-8906
        (C) TELEX: 200154

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 1:

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(i) SEQUENCE CHARACTERISTICS:

    (A) LENGTH: 2924
    (B) TYPE: nucleic acid
    (C) STRANDEDNESS: double
    (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
GACGCCGACG ATG AAG ACA CCG TGG AAG GTT CTT CTG GGA CTG CTG GGT        49
           Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly
            1               5                   10

GCT GCT GCG CTT GTC ACC ATC ATC ACC GTG CCC GTG GTT CTG CTG AAC        97
Ala Ala Ala Leu Val Thr Ile Ile Thr Val Pro Val Val Leu Leu Asn
        15              20                  25

AAA GGC ACA GAT GAT GCT ACA GCT GAC AGT CGC AAA ACT TAC ACT CTA       145
Lys Gly Thr Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu
    30              35                  40                  45

ACT GAT TAC TTA AAA AAT ACT TAT AGA CTG AAG TTA TAC TCC TTA AGA       193
Thr Asp Tyr Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg
                50                  55                  60

TGG ATT TCA GAT CAT GAA TAT CTC TAC AAA CAA GAA AAT AAT ATC TTG       241
Trp Ile Ser Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu
            65                  70                  75

GTA TTC AAT GCT GAA TAT GGA AAC AGC TCA GTT TTC TTG GAG AAC AGT       289
Val Phe Asn Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser
        80                  85                  90
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```
ACA TTT GAT GAG TTT GGA CAT TCT ATC AAT GAT TAT TCA ATA TCT CCT    337
Thr Phe Asp Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro
    95                  100                 105

GAT GGG CAG TTT ATT CTC TTA GAA TAC AAC TAC GTG AAG CAA TGG AGG    385
Asp Gly Gln Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg
110                 115                 120                 125

CAT TCC TAC ACA GCT TCA TAT GAC ATT TAT GAT TTA AAT AAA AGG CAG    433
His Ser Tyr Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln
                130                 135                 140

CTG ATT ACA GAA GAG AGG ATT CCA AAC AAC ACA CAG TGG GTC ACA TGG    481
Leu Ile Thr Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp
            145                 150                 155

TCA CCA GTG GGT CAT AAA TTG GCA TAT GTT TGG AAC AAT GAC ATT TAT    529
Ser Pro Val Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr
            160                 165                 170

GTT AAA ATT GAA CCA AAT TTA CCA AGT TAC AGA ATC ACA TGG ACG GGG    577
Val Lys Ile Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly
        175                 180                 185

AAA GAA GAT ATA ATA TAT AAT GGA ATA ACT GAC TGG GTT TAT GAA GAG    625
Lys Glu Asp Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu
190                 195                 200                 205

GAA GTC TTC AGT GCC TAC TCT GCT CTG TGG TGG TCT CCA AAC GGC ACT    673
Glu Val Phe Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn Gly Thr
            210                 215                 220

TTT TTA GCA TAT GCC CAA TTT AAC GAC ACA GAA GTC CCA CTT ATT GAA    721
Phe Leu Ala Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu
            225                 230                 235

TAC TCC TTC TAC TCT GAT GAG TCA CTG CAG TAC CCA AAG ACT GTA CGG    769
Tyr Ser Phe Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg
            240                 245                 250

GTT CCA TAT CCA AAG GCA GGA GCT GTG AAT CCA ACT GTA AAG TTC TTT    817
Val Pro Tyr Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys Phe Phe
    255                 260                 265

GTT GTA AAT ACA GAC TCT CTC AGC TCA GTC ACC AAT GCA ACT TCC ATA    865
Val Val Asn Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile
270                 275                 280                 285

CAA ATC ACT GCT CCT GCT TCT ATG TTG ATA GGG GAT CAC TAC TTG TGT    913
Gln Ile Thr Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys
            290                 295                 300
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```
GAT GTG ACA TGG GCA ACA CAA GAA AGA ATT TCT TTG CAG TGG CTC AGG     961
Asp Val Thr Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp Leu Arg
        305             310                 315

AGG ATT CAG AAC TAT TCG GTC ATG GAT ATT TGT GAC TAT GAT GAA TCC    1009
Arg Ile Gln Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser
        320             325                 330

AGT GGA AGA TGG AAC TGC TTA GTG GCA CGG CAA CAC ATT GAA ATG AGT    1057
Ser Gly Arg Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser
        335             340                 345

ACT ACT GGC TGG GTT GGA AGA TTT AGG CCT TCA GAA CCT CAT TTT ACC    1105
Thr Thr Gly Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr
350             355                 360                 365

CTT GAT GGT AAT AGC TTC TAC AAG ATC ATC AGC AAT GAA GAA GGT TAC    1153
Leu Asp Gly Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr
            370             375                 380

AGA CAC ATT TGC TAT TTC CAA ATA GAT AAA AAA GAC TGC ACA TTT ATT    1201
Arg His Ile Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile
        385             390                 395

ACA AAA GGC ACC TGG GAA GTC ATC GGG ATA GAA GCT CTA ACC AGT GAT    1249
Thr Lys Gly Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp
        400             405                 410

TAT CTA TAC TAC ATT AGT AAT GAA TAT AAA GGA ATG CCA GGA GGA AGG    1297
Tyr Leu Tyr Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly Gly Arg
    415             420                 425

AAT CTT TAT AAA ATC CAA CTT AGT GAC TAT ACA AAA GTG ACA TGC CTC    1345
Asn Leu Tyr Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu
430             435                 440                 445

AGT TGT GAG CTG AAT CCG GAA AGG TGT CAG TAC TAT TCT GTG TCA TTC    1393
Ser Cys Glu Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe
            450             455                 460

AGT AAA GAG GCG AAG TAT TAT CAG CTG AGA TGT TCC GGT CCT GGT CTG    1441
Ser Lys Glu Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu
        465             470                 475

CCC CTC TAT ACT CTA CAC AGC AGC GTG AAT GAT AAA GGG CTG AGA GTC    1489
Pro Leu Tyr Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val
        480             485                 490

CTG GAA GAC AAT TCA GCT TTG GAT AAA ATG CTG CAG AAT GTC CAG ATG    1537
Leu Glu Asp Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met
    495             500                 505
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```
CCC TCC AAA AAA CTG GAC TTC ATT ATT TTG AAT GAA ACA AAA TTT TGG    1585
Pro Ser Lys Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp
510             515             520             525

TAT CAG ATG ATC TTG CCT CCT CAT TTT GAT AAA TCC AAG AAA TAT CCT    1633
Tyr Gln Met Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro
530             535             540

CTA CTA TTA GAT GTG TAT GCA GGC CCA TGT AGT CAA AAA GCA GAC ACT    1681
Leu Leu Leu Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr
545             550             555

GTC TTC AGA CTG AAC TGG GCC ACT TAC CTT GCA AGC ACA GAA AAC ATT    1729
Val Phe Arg Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile
560             565             570

ATA GTA GCT AGC TTT GAT GGC AGA GGA AGT GGT TAC CAA GGA GAT AAG    1777
Ile Val Ala Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys
575             580             585

ATC ATG CAT GCA ATC AAC AGA AGA CTG GGA ACA TTT GAA GTT GAA GAT    1825
Ile Met His Ala Ile Asn Arg Arg Leu Gly Thr Phe Glu Val Glu Asp
590             595             600             605

CAA ATT GAA GCA GCC AGA CAA TTT TCA AAA ATG GGA TTT GTG GAC AAC    1873
Gln Ile Glu Ala Ala Arg Gln Phe Ser Lys Met Gly Phe Val Asp Asn
610             615             620

AAA CGA ATT GCA ATT TGG GGC TGG TCA TAT GGA GGG TAC GTA ACC TCA    1921
Lys Arg Ile Ala Ile Trp Gly Trp Ser Tyr Gly Gly Tyr Val Thr Ser
625             630             635

ATG GTC CTG GGA TCA GGA AGT GGC GTG TTC AAG TGT GGA ATA GCC GTG    1969
Met Val Leu Gly Ser Gly Ser Gly Val Phe Lys Cys Gly Ile Ala Val
640             645             650

GCG CCT GTA TCC CGG TGG GAG TAC TAT GAC TCA GTG TAC ACA GAA CGT    2017
Ala Pro Val Ser Arg Trp Glu Tyr Tyr Asp Ser Val Tyr Thr Glu Arg
655             660             665

TAC ATG GGT CTC CCA ACT CCA GAA GAC AAC CTT GAC CAT TAC AGA AAT    2065
Tyr Met Gly Leu Pro Thr Pro Glu Asp Asn Leu Asp His Tyr Arg Asn
670             675             680             685

TCA ACA GTC ATG AGC AGA GCT GAA AAT TTT AAA CAA GTT GAG TAC CTC    2113
Ser Thr Val Met Ser Arg Ala Glu Asn Phe Lys Gln Val Glu Tyr Leu
690             695             700

CTT ATT CAT GGA ACA GCA GAT GAT AAC GTT CAC TTT CAG CAG TCA GCT    2161
Leu Ile His Gly Thr Ala Asp Asp Asn Val His Phe Gln Gln Ser Ala
705             710             715
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```
CAG ATC TCC AAA GCC CTG GTC GAT GTT GGA GTG GAT TTC CAG GCA ATG   2209
Gln Ile Ser Lys Ala Leu Val Asp Val Gly Val Asp Phe Gln Ala Met
        720                 725                 730

TGG TAT ACT GAT GAA GAC CAT GGA ATA GCT AGC AGC ACA GCA CAC CAA   2257
Trp Tyr Thr Asp Glu Asp His Gly Ile Ala Ser Ser Thr Ala His Gln
    735                 740                 745

CAT ATA TAT ACC CAC ATG AGC CAC TTC ATA AAA CAA TGT TTC TCT TTA   2305
His Ile Tyr Thr His Met Ser His Phe Ile Lys Gln Cys Phe Ser Leu
750                 755                 760                 765

CCT TAGCACCTCA AAATACCATG CCATTTAAAG CTTATTAAAA CTCATTTTTG         2358
Pro

TTTTCATTAT CTCAAAACTG CACTGTCAAG ATGATGATGA TCTTTAAAAT ACACACTCAA   2418

ATCAAGAAAC TTAAGGTTAC CTTTGTTCCC AAATTTCATA CCTATCATCT TAAGTAGGGA   2478

CTTCTGTCTT CACAACAGAT TATTACCTTA CAGAAGTTTG AATTATCCGG TCGGGTTTTA   2538

TTGTTTAAAA TCATTTCTGC ATCAGCTGCT GAAACAACAA ATAGGAATTG TTTTTATGGA   2598

GGCTTTGCAT AGATTCCCTG AGCAGGATTT TAATCTTTTT CTAACTGGAC TGGTTCAAAT   2658

GTTGTTCTCT TCTTTAAAGG GATGGCAAGA TGTGGGCAGT GATGTCACTA GGGCAGGGAC   2718

AGGATAAGAG GGATTAGGGA GAGAAGATAG CAGGGCATGG CTGGGAACCC AAGTCCAAGC   2778

ATACCAACAC GACCAGGCTA CTGTCAGCTC CCCTCGGAGA AAACTGTGCA GTCTGCGTGT   2838

GAACAGCTCT TCTCCTTTAG AGCACAATGG ATCTCGAGGG ATCTTCCATA CCTACCAGTT   2898

CTGCGCCTCG AGGCCGCGAC TCTAGA                                        2924
```

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 2:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 7
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY:

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Thr Pro Trp Lys Val Leu Ile
  1               5
```

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 3:

   (i) SEQUENCE CHARACTERISTICS:

           (A) LENGTH: 11
           (B) TYPE: amino acid
           (C) STRANDEDNESS:
           (D) TOPOLOGY:

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Pro Val Val Leu Leu Asn Lys Gly Thr Asp Asp
1   5   10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 4:

   (i) SEQUENCE CHARACTERISTICS:

           (A) LENGTH: 6
           (B) TYPE: amino acid
           (C) STRANDEDNESS:
           (D) TOPOLOGY:

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Lys Gly Leu Leu Gly
1   5

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 5:

   (i) SEQUENCE CHARACTERISTICS:

           (A) LENGTH: 13
           (B) TYPE: amino acid
           (C) STRANDEDNESS:
           (D) TOPOLOGY:

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly
1   5   10

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 6:

    (i) SEQUENCE CHARACTERISTICS:

             (A) LENGTH: 30
             (B) TYPE: nucleic acid
             (C) STRANDEDNESS: single
             (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

ACGCCGACGA TGAAGGGACT GCTGGGTGCT   30

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 7:

    (i) SEQUENCE CHARACTERISTICS:

             (A) LENGTH: 10
             (B) TYPE: amino acid
             (C) STRANDEDNESS:
             (D) TOPOLOGY:

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ile Ile Thr Val Ala Thr Ala Asp Ser Arg
1   5   10

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 8:

    (i) SEQUENCE CHARACTERISTICS:

             (A) LENGTH: 21
             (B) TYPE: amino acid
             (C) STRANDEDNESS:
             (D) TOPOLOGY:

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile Ile Thr Val Pro Val Val Leu Leu Asn Lys Gly Thr Asp Asp Ala
1   5   10   15

Thr Ala Asp Ser Arg
            20

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 9:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 30
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

ACCATCATCA CCGTGGCTAC AGCTGACAGT   30

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 10:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 22
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTACGTATCA TTAGCTATTC GA   22

(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 11:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 603
        (B) TYPE: amino acid
        (C) STRANDEDNESS: N/A
        (D) TOPOLOGY: N/A

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Lys Thr Pro Trp Lys Val Leu Leu Gly Leu Leu Gly Ala Ala Ala
 1   5   10   15

Leu Val Thr Ile Ile Thr Val Pro Val Leu Leu Asn Lys Gly Thr
            20   25   30

Asp Asp Ala Thr Ala Asp Ser Arg Lys Thr Tyr Thr Leu Thr Asp Tyr
        35   40   45

Leu Lys Asn Thr Tyr Arg Leu Lys Leu Tyr Ser Leu Arg Trp Ile Ser
    50   55   60

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```
Asp His Glu Tyr Leu Tyr Lys Gln Glu Asn Asn Ile Leu Val Phe Asn
65                  70              75                      80

Ala Glu Tyr Gly Asn Ser Ser Val Phe Leu Glu Asn Ser Thr Phe Asp
                85                  90                      95

Glu Phe Gly His Ser Ile Asn Asp Tyr Ser Ile Ser Pro Asp Gly Gln
            100              105              110

Phe Ile Leu Leu Glu Tyr Asn Tyr Val Lys Gln Trp Arg His Ser Tyr
        115              120                  125

Thr Ala Ser Tyr Asp Ile Tyr Asp Leu Asn Lys Arg Gln Leu Ile Thr
    130              135              140

Glu Glu Arg Ile Pro Asn Asn Thr Gln Trp Val Thr Trp Ser Pro Val
145              150                  155                  160

Gly His Lys Leu Ala Tyr Val Trp Asn Asn Asp Ile Tyr Val Lys Ile
            165              170                  175

Glu Pro Asn Leu Pro Ser Tyr Arg Ile Thr Trp Thr Gly Lys Glu Asp
            180              185              190

Ile Ile Tyr Asn Gly Ile Thr Asp Trp Val Tyr Glu Glu Glu Val Phe
        195              200              205

Ser Ala Tyr Ser Ala Leu Trp Trp Ser Pro Asn Gly Thr Phe Leu Ala
    210              215              220

Tyr Ala Gln Phe Asn Asp Thr Glu Val Pro Leu Ile Glu Tyr Ser Phe
225              230              235              240

Tyr Ser Asp Glu Ser Leu Gln Tyr Pro Lys Thr Val Arg Val Pro Tyr
            245              250                  255

Pro Lys Ala Gly Ala Val Asn Pro Thr Val Lys Phe Phe Val Val Asn
            260              265              270

Thr Asp Ser Leu Ser Ser Val Thr Asn Ala Thr Ser Ile Gln Ile Thr
        275              280              285

Ala Pro Ala Ser Met Leu Ile Gly Asp His Tyr Leu Cys Asp Val Thr
    290              295              300

Trp Ala Thr Gln Glu Arg Ile Ser Leu Gln Trp Leu Arg Arg Ile Gln
305              310              315              320

Asn Tyr Ser Val Met Asp Ile Cys Asp Tyr Asp Glu Ser Ser Gly Arg
325              330              335              340
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```
Trp Asn Cys Leu Val Ala Arg Gln His Ile Glu Met Ser Thr Thr Gly
          345             350             355

Trp Val Gly Arg Phe Arg Pro Ser Glu Pro His Phe Thr Leu Asp Gly
          360             365             370

Asn Ser Phe Tyr Lys Ile Ile Ser Asn Glu Glu Gly Tyr Arg His Ile
          375             380             385

Cys Tyr Phe Gln Ile Asp Lys Lys Asp Cys Thr Phe Ile Thr Lys Gly
 390             395             400             405

Thr Trp Glu Val Ile Gly Ile Glu Ala Leu Thr Ser Asp Tyr Leu Tyr
              410             415             420

Tyr Ile Ser Asn Glu Tyr Lys Gly Met Pro Gly Gly Arg Asn Leu Tyr
          425             430             435

Lys Ile Gln Leu Ser Asp Tyr Thr Lys Val Thr Cys Leu Ser Cys Glu
          440             445             450

Leu Asn Pro Glu Arg Cys Gln Tyr Tyr Ser Val Ser Phe Ser Lys Glu
     455             460             465

Ala Lys Tyr Tyr Gln Leu Arg Cys Ser Gly Pro Gly Leu Pro Leu Tyr
 470             475             480             485

Thr Leu His Ser Ser Val Asn Asp Lys Gly Leu Arg Val Leu Glu Asp
              490             495             500

Asn Ser Ala Leu Asp Lys Met Leu Gln Asn Val Gln Met Pro Ser Lys
              505             510             515

Lys Leu Asp Phe Ile Ile Leu Asn Glu Thr Lys Phe Trp Tyr Gln Met
          520             525             530

Ile Leu Pro Pro His Phe Asp Lys Ser Lys Lys Tyr Pro Leu Leu Leu
     535             540             545

Asp Val Tyr Ala Gly Pro Cys Ser Gln Lys Ala Asp Thr Val Phe Arg
 550             555             560             565

Leu Asn Trp Ala Thr Tyr Leu Ala Ser Thr Glu Asn Ile Ile Val Ala
 570             575             580             585

Ser Phe Asp Gly Arg Gly Ser Gly Tyr Gln Gly Asp Lys Ile Met His
          590             595             600

Ala
```

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(2) INFORMATION FOR SEQUENCE IDENTIFICATION NUMBER: 12:

    (i) SEQUENCE CHARACTERISTICS:

        (A) LENGTH: 7
        (B) TYPE: amino acid
        (C) STRANDEDNESS:
        (D) TOPOLOGY:

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Gly Asp Lys Ile Met His Ala
 1   5

What is claimed is:

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Claims

1. A polypeptide fragment of CD26 having an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 2 (Δ3-9).

5   2. A nucleic acid encoding the polypeptide of claim 1.

3. A polypeptide fragment of CD26 having an amino acid sequence substantially identical to the amino acid sequence of SEQ ID NO: 3 (Δ24-34).

10   4. A nucleic acid encoding the polypeptide of claim 3.

5. The polypeptide of claim 1, wherein said polypeptide has an amino acid sequence identical to the amino acid sequence of SEQ ID NO: 2.

15   6. The polypeptide of claim 3, wherein said polypeptide has an amino acid sequence identical to the amino acid sequence of SEQ ID NO: 3.

7. The polypeptide of claim 1, said polypeptide being soluble under physiological conditions.

20   8. The polypeptide of claim 3, said polypeptide being soluble under physiological conditions.

9. The polypeptide of claim 1, said polypeptide being substantially pure.

10. The polypeptide of claim 3, said polypeptide
25 being substantially pure.

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11. A plasmid comprising the nucleic acid of any of claims 2 or 4.

12. The plasmid of claim 11, said plasmid further comprising an expression control sequence capable of
5 directing expression of said polypeptide.

13. A polypeptide fragment of CD26 or analogs thereof capable of disrupting the naturally occurring binding interaction between CD45 and CD26.

14. A method for screening candidate compounds to
10 identify compounds capable of inhibiting the binding of CD26 to CD45, said method comprising the steps of:
   (a) providing a first and a second sample of cells expressing both CD26 and CD45;
   (b) incubating said first sample in the presence
15 of a candidate compound;
   (c) incubating said second sample in the absence of said candidate compound;
   (d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD26
20 antibody;
   (e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said antibody; and
   (f) determining whether the amount of CD45 present
25 in said first immunoprecipitate is less than the amount of CD45 present in said second immunoprecipitate, the presence of a lesser amount of CD45 in said first immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said
30 binding.

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15.   A method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to CD45, said method comprising the steps of:

(a) providing a first and a second sample of cells
5 expressing both CD26 and CD45;

(b) incubating said first sample in the presence of a candidate compound;

(c) incubating said second sample in the absence of said candidate compound;
10   (d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD45 antibody;

(e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said
15 antibody; and

(f) determining whether the amount of CD26 present in said first immunoprecipitate is less than the amount of CD26 present in said second immunoprecipitate, the presence of a lesser amount of CD26 in said first
20 immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said binding.

16.   A monoclonal antibody which, when contacted under physiological conditions with a cell expressing
25 CD26 and CD45, interferes with the association of said CD26 and CD45.

17.   A method comprising:

(a) providing a cell which expresses CD45 on its surface; and
30   (b) introducing into said cell a nucleic acid encoding CD26, such that said cell expresses CD26 on its surface.

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18.   A method comprising:

(a) providing a cell which expresses CD26 on its surface; and

(b) introducing into said cell a nucleic acid
5 encoding CD45, such that said cell expresses CD45 on its surface.

19.   A cell transfected with a nucleic acid encoding CD26, said cell expressing both CD26 and CD45 on its surface.

10   20.   A cell transfected with a nucleic acid encoding CD45, said cell expressing both CD26 and CD45 on its surface.

21.   The cell of claim 19, wherein said cell is a Jurkat cell.

15   22.   The cell of claim 20, wherein said cell is a Jurkat cell.

23.   A method comprising:

(a) providing a cell which expresses neither CD26 nor CD45 on its surface; and
20   (b) transfecting said cell with a nucleic acid encoding CD26 and a nucleic acid encoding CD45.

24.   A method of generating a hybridoma cell, said method comprising:

(a) providing a cell transfected with nucleic acid
25 encoding CD26, such that said cell expresses CD26 on its surface;

(b) using said cell as an antigen to induce an immune response in a subject animal; and

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(c) fusing a B lymphocyte from said subject animal with a cell from an immortal cell line to produce a hybridoma cell.

25. A hybridoma cell generated by the method of claim 24, wherein said hybridoma cell produces a monoclonal antibody specific for CD26.

26. A cell-free preparation of CD26, or a fragment thereof, complexed with CD45, or a fragment thereof.

27. A polypeptide fragment of CD26 or analog thereof capable of disrupting the naturally-occurring binding interaction between p43 and CD26.

28. A method for screening candidate compounds to identify compounds capable of inhibiting the binding of CD26 to p43, said method comprising the steps of:

(a) providing a first and a second sample of cells expressing both CD26 and p43;

(b) incubating said first sample in the presence of a candidate compound;

(c) incubating said second sample in the absence of said candidate compound;

(d) generating a first immunoprecipitate by adding to said first sample a first aliquot of an anti-CD26 antibody;

(e) generating a second immunoprecipitate by adding to said second sample a second aliquot of said antibody; and

(f) determining whether the amount of p43 present in said first immunoprecipitate is less than the amount of p43 present in said second immunoprecipitate, the presence of a lesser amount of p43 in said first

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immunoprecipitate than in said second immunoprecipitate indicating that said candidate compound inhibits said binding.

29. A purified preparation of p43.

5   30. A method of detecting CD26 mRNA in a cell, said method comprising the steps of:

(a) providing a biological sample comprising mRNA of a cell;

(b) contacting said sample with a single-stranded 10 nucleic acid probe comprising a segment of the sense strand of SEQ ID NO: 1 at least 8 nucleotides in length; and

(c) detecting hybridization of said probe with said sample, said hybridization indicating the presence 15 of CD26 mRNA in said cell.

31. A fragment of CD26 in which at least one of the amino acids in the segment Gly627-Gly631 is deleted.

32. The fragment of claim 31, wherein all of said segment is deleted.

20   33. The fragment of claim 32, wherein said fragment has the amino acid sequence shown in SEQ ID NO: 8.

34. A polypeptide fragment of CD26 lacking residues 1-34 of intact CD26.

25   35. The polypeptide fragment of claim 34, wherein said fragment additionally lacks residue 35.

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36.   The polypeptide fragment of claim 35, wherein said fragment additionally lacks residue 36.

37.   The polypeptide fragment of claim 36, wherein said fragment additionally lacks residue 37.

```
  1: GACGCCGACGATGAAGACACCGTGGAAGGTTCTTCTGGGACTGCTGGGGTGCTGCTGGGCTTGTCAC
  1:                         M  K  T  P  W  K  V  L  L  G  L  L  G  A  A  A  L  V  T

 67: CATCATCACCGTGCCGTGGTTCTGGTTCTGCTGAACAAAGGCACAGATGATGCTACAGCTGACAGTCGCAAAA
 20:  I  I  T  V  P  V  V  L  L  N  K  G  T  D  D  A  T  A  D  S  R  K

135: CTTACACTCTAACTGATTACTTAAAAAATACTTATAGACTGAAGTTATACTCCTTAAGATGGATTCA
 42:  T  Y  L  T  D  Y  L  K  N  T  Y  R  L  K  L  Y  S  L  R  W  I  S

203: GATCATGAATATCTCTACAAACAAGAAAATAATATCTTGGTATTCAATGCTGAATATGGAAACAGCTC
 65:  D  N  E  Y  L  Y  K  Q  E  N  N  I  L  V  F  N  A  E  Y  G  N  S

270: AGTTTCTTGGAGAACAGTACATTTGATGAGTTTGGACATTCTATCAATGATTATTCAATATCTCCTG
 88:  V  F  L  E  N  S  T  F  D  E  F  G  H  S  I  N  D  Y  S  I  S  P

337: ATGGGCAGTTATTCTTCTTAGAATACAACTACGTGAAGCAATGGAGGCATTCCTACACAGCTTCATAT
110:  D  G  Q  F  I  L  L  E  Y  N  Y  V  K  Q  W  R  H  S  Y  T  A  S  Y

404: GACATTTATGATTAAATAAAAGGCAGCTGATTACAGAGAGAGGATCCAAACAACACACAGTGGGT
133:  D  I  Y  D  L  N  K  R  Q  L  I  T  E  E  R  I  P  N  N  T  Q  W  V

571: CACATGGTCACCAGTGGGTCATAAATTGGCATATAAATGACATTTATGTTAAAATTGAAC
156:  T  W  S  P  V  G  H  K  L  A  Y  V  W  N  N  D  I  Y  V  K  I  E

638: CAAATTTACCAGTTACAGAATCACATGGACGGGGAAAGAAGAGATATAATATAATGGAATAACTGAC
178:  P  N  L  P  S  Y  R  I  T  W  T  G  K  E  D  I  I  Y  N  G  I  T  D

705: TGGGTTTATGAAGAGGAAGTCTTCAGTGCCTACTCTGCTCTGTGGTGGTCTCCAAACGGCACTTTTT
201:  W  V  Y  E  E  E  V  F  S  A  Y  S  A  L  W  W  S  P  N  G  T  F  L
```

FIG. 1A

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```
872:  AGCATATGCCCAATTTAACGACACAGAAGTCCCCACTTATTGAATACTCCTTCTACTCTGATGAGTCAC
224:  A  Y  A  Q  F  N  D  T  E  V  P  L  I  E  Y  S  F  Y  S  D  E  S

939:  TGCAGTACCACCAAAGACTGTACGGGTTCCATATCCAAAGGCAGGAGCTGTGAATCCAACTGTAAAGTTC
246:  L  Q  Y  P  K  T  V  R  V  P  Y  P  K  A  G  A  V  N  P  T  V  K  F

1006: TTTGTTGTAAATACAGACTCTCTCAGTCACCAATGCAACTTCCATACAAATCACTGCTCCTGC
269:  F  V  V  N  T  D  S  L  S  S  V  T  N  A  T  S  I  Q  I  T  A  P  A

1073: TTCTATGTTGATAGGGGATCACTACTTGTGTGTGATGTGGCAACACAAGAAAGAATTTCTTTGC
292:  S  M  L  I  G  D  S  Y  L  C  D  V  T  W  A  T  Q  E  R  I  S  L

1140: AGTGGCTCAGGAGGATTCAGAACTATTCGGTCGGTCATGGATATTTGTGACTATGATGAATCCAGTGGAAGA
314:  Q  W  L  R  R  I  Q  N  Y  S  V  M  D  I  C  D  Y  D  E  S  S  G  R

1207: TGGAACTGCTTAGTGGCCACGGCAACACCATTGAAATGAGTACTACTGGCTGGGTTGGAAGATTTAGGCC
337:  W  N  C  L  V  A  R  Q  H  I  E  M  S  T  T  G  W  V  G  R  F  R  P

1274: TTCAGAACCTCATTTTACCCTTGATGGTAATAGCTTCTTCCTACAGATCATCAGCAATGAAGAAGGTTACA
360:  S  E  P  H  F  T  L  D  G  N  S  F  Y  K  I  I  S  N  E  E  G  Y

1341: GACACATTTGCTATTCCAAATAGATAAAAAGACTGCACATTTATTACAAAGGCACCTGGGAAGTC
382:  R  H  I  C  Y  F  Q  I  D  K  K  D  C  T  F  I  T  K  G  T  W  E  V

1408: ATCGGGATAGAGCTCTAACCAGTGATTATCTATACTACTACATTAGTAATGAATATAAAGGAATGCCAGG
405:  I  G  I  E  A  L  T  S  D  Y  L  Y  Y  I  S  N  E  Y  K  G  M  P  G

1475: AGGAAGGAATCTTTATAAAATCCAACTTAGTGACTATACAAAAGTGACACTGCCTCAGTTGTGAGCTGAA
429:  G  R  N  L  Y  K  I  Q  L  S  D  Y  T  K  V  T  L  S  C  E  L  N

1542: TCCGGAAAGGTGTCAGTACTATTCTGTGTCATTCAGTAAAGAGGCGAAGTATTATCAGCTGAGATGTTC
452:  P  E  R  C  Q  Y  Y  S  V  S  F  S  K  E  A  K  Y  Y  Q  L  R  C  S
```

FIG. 1B

1609: CGGTCCTGGTCTGCCCCTCTATACTCTACACAGCAGCGTGAATGATAAAGGGCTGAGAGTCCTGGAAGA
475:    G P G L P L Y T L H S S V N D K G L R V L E D

1676: CAATTCAGCTTTGGATAAAATGCTGCAGAATGTCCAGATGCCCTCCAAAAAACTGGACTTCATTATTT
498:    N S A L D K M L Q N V Q M P S K K L D F I I L

1743: GAATGAAACAAAATTTGGTATCAGATGATCTTGCCTCCTCATTTGATAAATCCAAGAAATATCCTCT
521:    N E T K F W Y Q M I L P P H-F D K S K _K_ Y P L

1810: ACTATTAGATGTGTATGCAGGCCCATGTAGTCAAAAAGCAGACACTGTCTTCAGACTGAACTGGGCCAC
544:    L L D V Y A G P C S Q K A D T V F R L N W A T
                                  *

1877: TTACCTTGCAAGCACAGAAAACATTATAGTAGCTAGCTTTGATGGCAGAGGAAGTGGTTACCAAGGAGA
567:    Y L A S T E N I I V A S F D G R G S G Y Q G D

1944: TAAGATCATGCATGCAATCAACAGAAGACTGGGAACATTGAAGTTGAAGATCAAATTGAAGCAGCCAG
590:    K I M H A I N R R L G T F E V E D Q I E A A R

2011: ACAATTTTCAAAAAATGGATTTGTGGACAACAAACGAATTGCAATTTGGGCTGGTCATATGGAGGGTA
613:    Q F S K M G F V D N K R I A I W G W S Y G G Y

2078: CGTAACCTCAATGGTCCTGGGATCAGGAAGTGGCGTGTTCAAGTGTGGAATAGCCGTGGCCCTGTATC
636:    V T S M V L G S G S G V F K C G I A V A P V S
                                         *

FIG. 1C

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2145: CCGGTGGGGAGTACTATGACTCAGTGTACACAGAACGTTACATGGGTCTCCCAACTCCCAGAAGACAACCT
659:   R W E Y Y D S V Y T E R Y M G L P T P E D N L

2212: TGACCATTACAGAAATTCAACAGTCATGAGCAGAGCTGAAAATTTAAACAAGTTGAGTACCTCCTTAT
682:   D H Y R N S T V M S R A E N F K Q V E Y L L I

2279: TCATGGAACAGCAGATGATAACGTTCACTTCAGCAGTCAGATCTCCAAAGCCCTGGTCGATGT
705:   H G T A D D N V H F Q Q S A Q I S K A L V D V

2346: TGGAGTGGATTCCAGGCAATGTGGTATACTGATGAAGACCATGGAATAGCTAGCAGCACAGCACACCA
728:   G V D F Q A M W Y T D E D H G I A S S T _A_ H Q

2413: ACATATATATACCCACATGAGCCACTTCATAAAACAATGTTTCTCTTACCTTAGCACCTCAAAATACC
751:   H I Y T H M S H F I K Q C F S L P *

2480: ATGCCATTTAAAGCTTATTAAAACTCATTTTGTTTTTCATTATCTCAAAACTGCACTGTCAAGATGATG
2547: ATGATCTTAAAATACACACTCAAATCAAGAAACTTAAGGTTACCTTTGTTCCCAAATTCATACCTAT
2614: CATCTTAAGTAGGGACTTCTGTCTTCCACACAGATTATTACCTTACAGAAGTTTGAATTATCCGGTCGG
2681: GTTTTATTGTTTAAAATCATTCTGCAGCAGGATTTAATCTTTTTTCTTGGTTTTATGGAGGC
2748: TTTGCATAGATCCCTGAGCAGGATTTGGGCCAAGATGTGGCAGTGATGTCACTAGGGACTGGGACAGG
2815: TTTAAAGGGATGGCAAGATGGCTGGGACTGGTTCAAATGTTGTTCTCTTC
2882: AGAAGATAGCAGGGCATGGCTGGGAACCCAAGCATACCAACACGACCAGCTACTGTCAGCTC
2919: CCCTCGGAGAAAACTGTGTGCAGTCTGCGTGTGAACAGCTCTTCTCCTTTAGAGCACAATGGATCTCGAGG
3016: GATCTTCCATACCTACCAGTTCTGCGCCCTCGAGGCCGCGACTCTAGA   3063
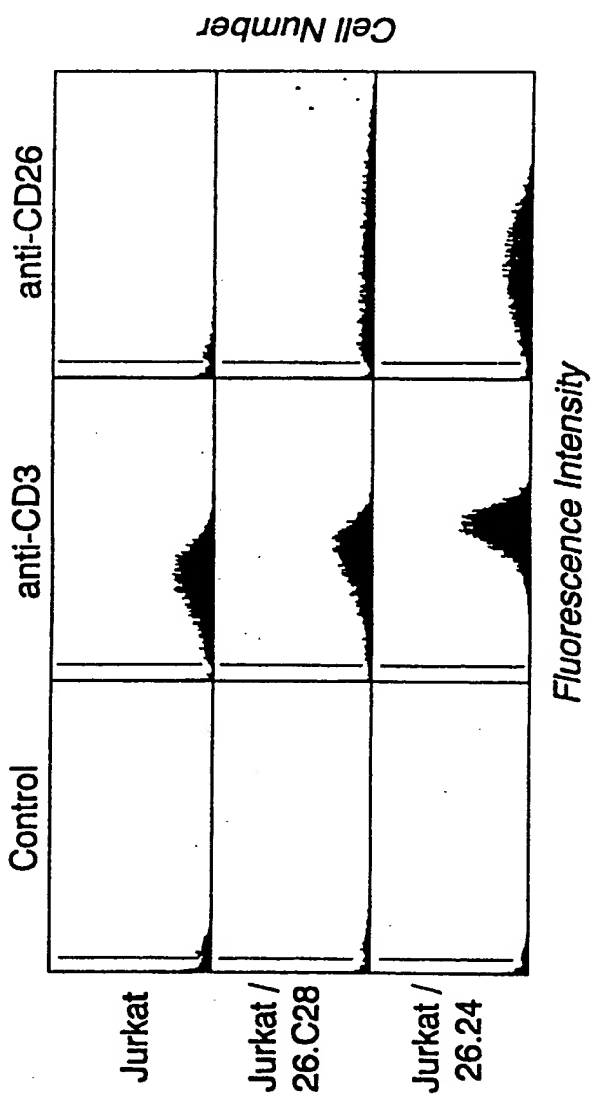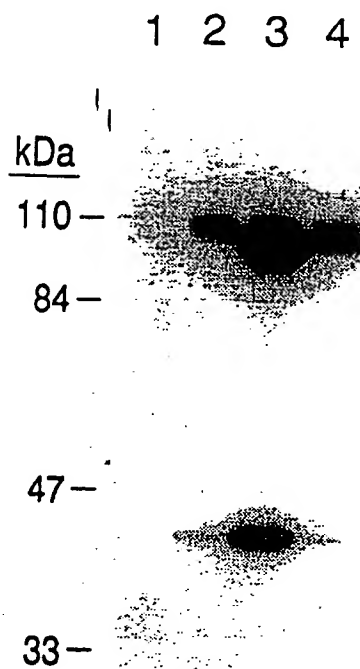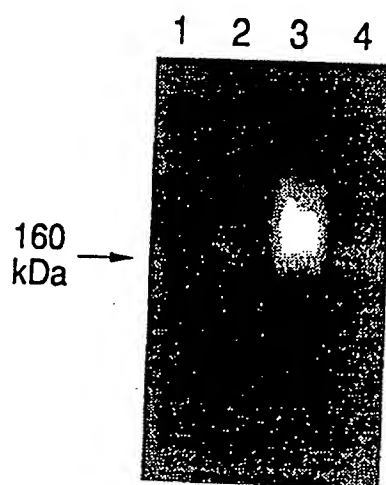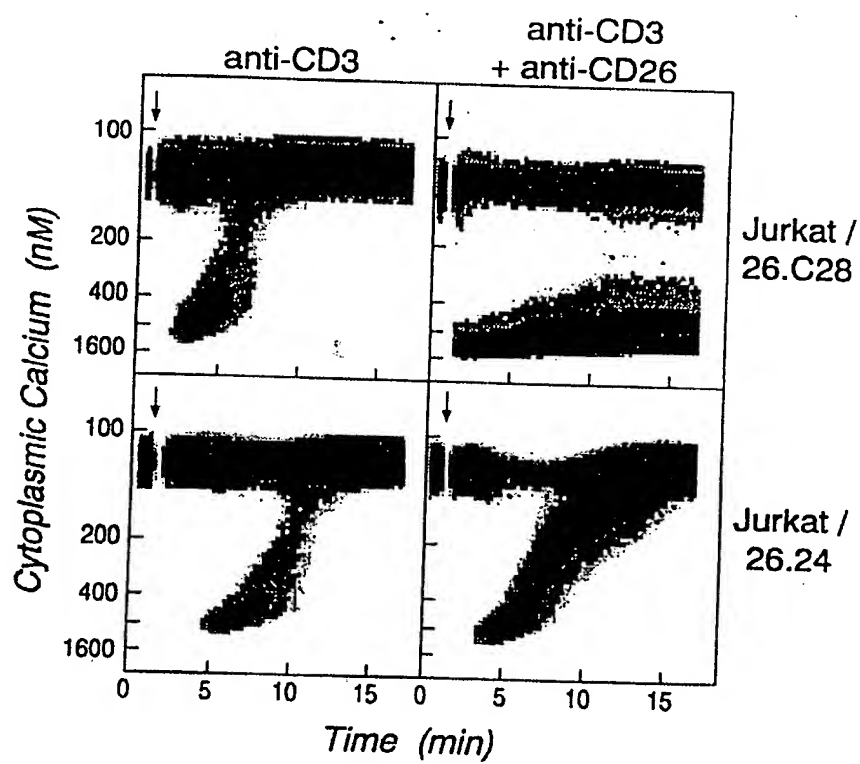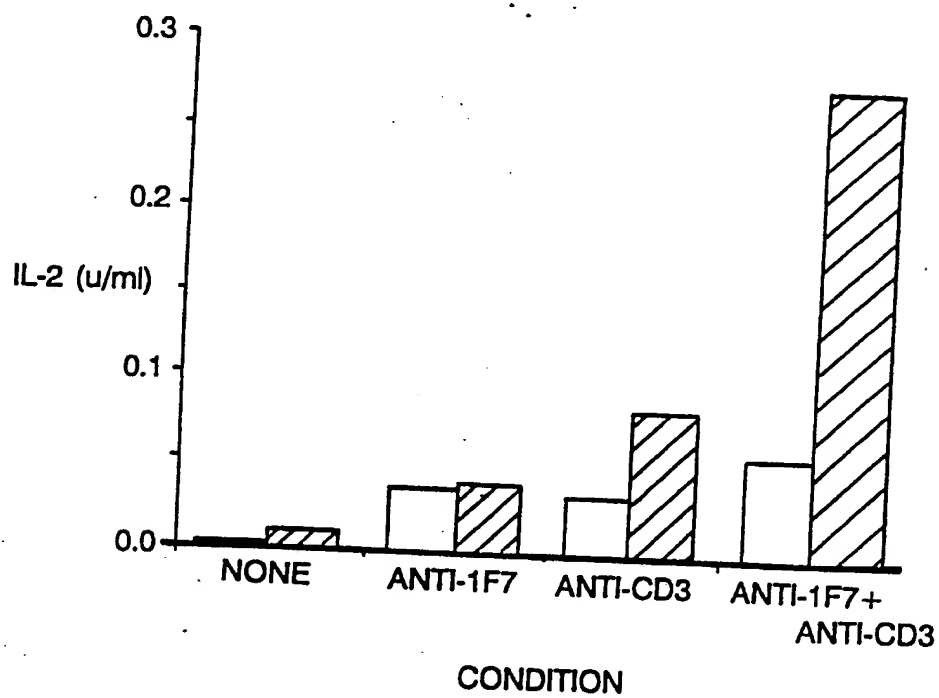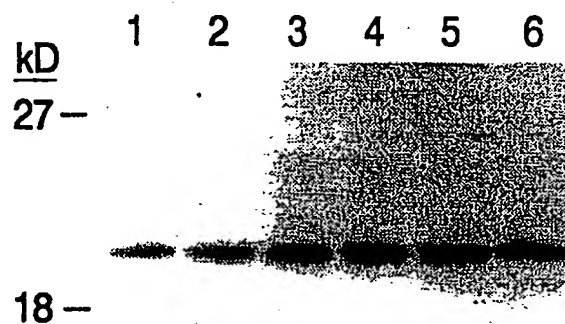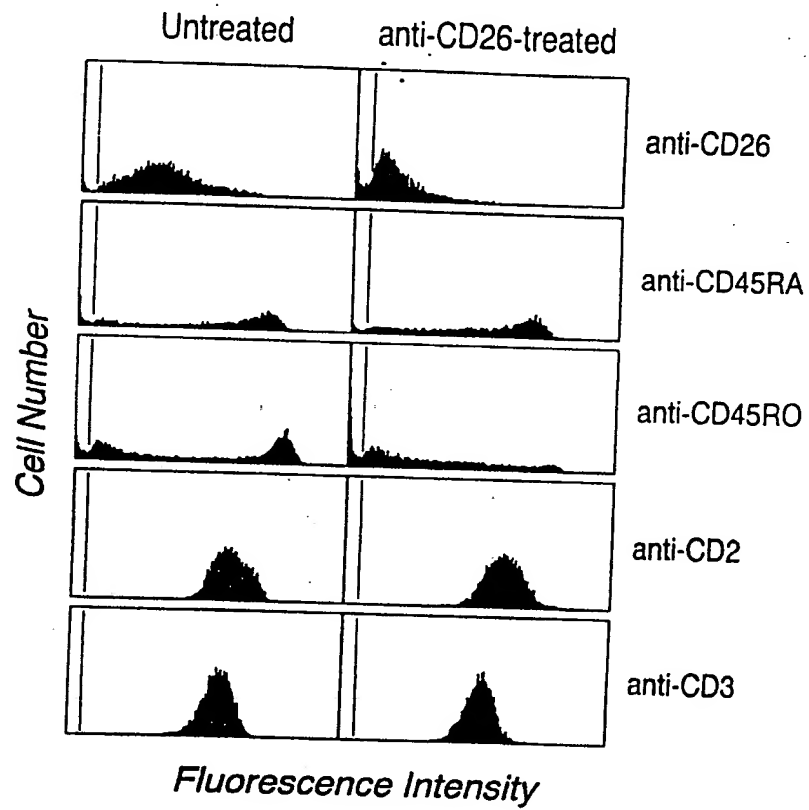
FIG. 1D

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FIG. 2

FIG. 3A

FIG. 3B

FIG. 4

FIG. 5

FIG. 6

FIG. 7

FIG. 8

FIG. 9

FIG. 10

FIG. 11

FIG. 12

CD26:

```
1
MK[TPWKVLL]GLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSL
                                                51
RWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNY
                                    101
VKQWRHSYTASYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNL
                    151
PSYRITWTGKEDIIYNGITDWVYEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSF
            201
YSDESLQYPKTVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYL
    251
CDVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPS
301         351
EPHFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISN
                            401
EYKGMPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLY
                    451
TLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKSKKY
            501
PLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKIMHAINRRLGT
    551
FEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGSGVFKCGIAVAPVSRWE
601         651
YYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQSAQIS
                    701
KALVDVGVDFQAMWYTDEDHGIASSTAHQHIYTHMSHFIKQCFSLP
            751
```

FIG. 13

D26:

1
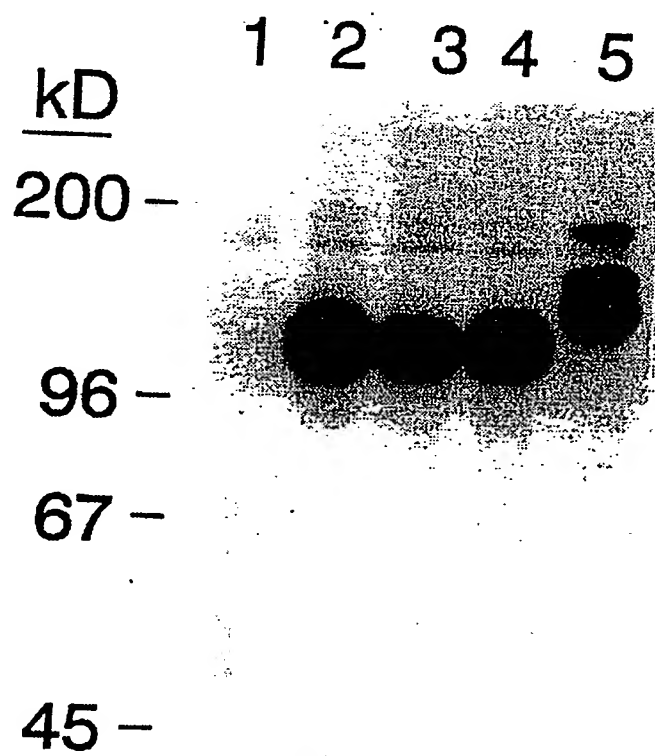MKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSL
51   101

RWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNY
                                151

VKQWRHSYTASYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNL
                                201

PSYRITWTGKEDIIYNGITDWVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSF
251

YSDESLQYPKTVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYL
301   351

CDVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPS
                                401

EPHFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISN
                                451

EYKGMPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLY
                                501

TLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKSKKY
551

PLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKIMHAINRRLGT
601   651

FEVEDQIEAARQFSKMGFVDNKRIAIWGWSYGGYVTSMVLGSGGVFKCGIAVAPVSRWE
                                701

YYDSVYTERYMGLPTPEDNLDHYRNSTVMSRAENFKQVEYLLIHGTADDNVHFQQSAQIS
751

KALVDVGVDFQAMWYTDEDHGIASSTAHQHIYTHMSHFIKQCFSLP

FIG. 14

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CD26:

```
1    MKTPWKVLLGLLGAAALVTIITVPVVLLNKGTDDATADSRKTYTLTDYLKNTYRLKLYSL
                                                               51

RWISDHEYLYKQENNILVFNAEYGNSSVFLENSTFDEFGHSINDYSISPDGQFILLEYNY
                                                               101

VKQWRHSYTASYDIYDLNKRQLITEERIPNNTQWVTWSPVGHKLAYVWNNDIYVKIEPNL
                                                               151

PSYRITWTGKEDIIYNGITDWVYEEEVFSAYSALWWSPNGTFLAYAQFNDTEVPLIEYSF
                                                               201

YSDESLQYPKTVRVPYPKAGAVNPTVKFFVVNTDSLSSVTNATSIQITAPASMLIGDHYL
                                                               251

301  CDVTWATQERISLQWLRRIQNYSVMDICDYDESSGRWNCLVARQHIEMSTTGWVGRFRPS
                                                               351

EPHFTLDGNSFYKIISNEEGYRHICYFQIDKKDCTFITKGTWEVIGIEALTSDYLYYISN
                                                               401

EYKGMPGGRNLYKIQLSDYTKVTCLSCELNPERCQYYSVSFSKEAKYYQLRCSGPGLPLY
                                                               451

TLHSSVNDKGLRVLEDNSALDKMLQNVQMPSKKLDFIILNETKFWYQMILPPHFDKSKKY
                                                               501

PLLLDVYAGPCSQKADTVFRLNWATYLASTENIIVASFDGRGSGYQGDKIMHA
                                                               551
```
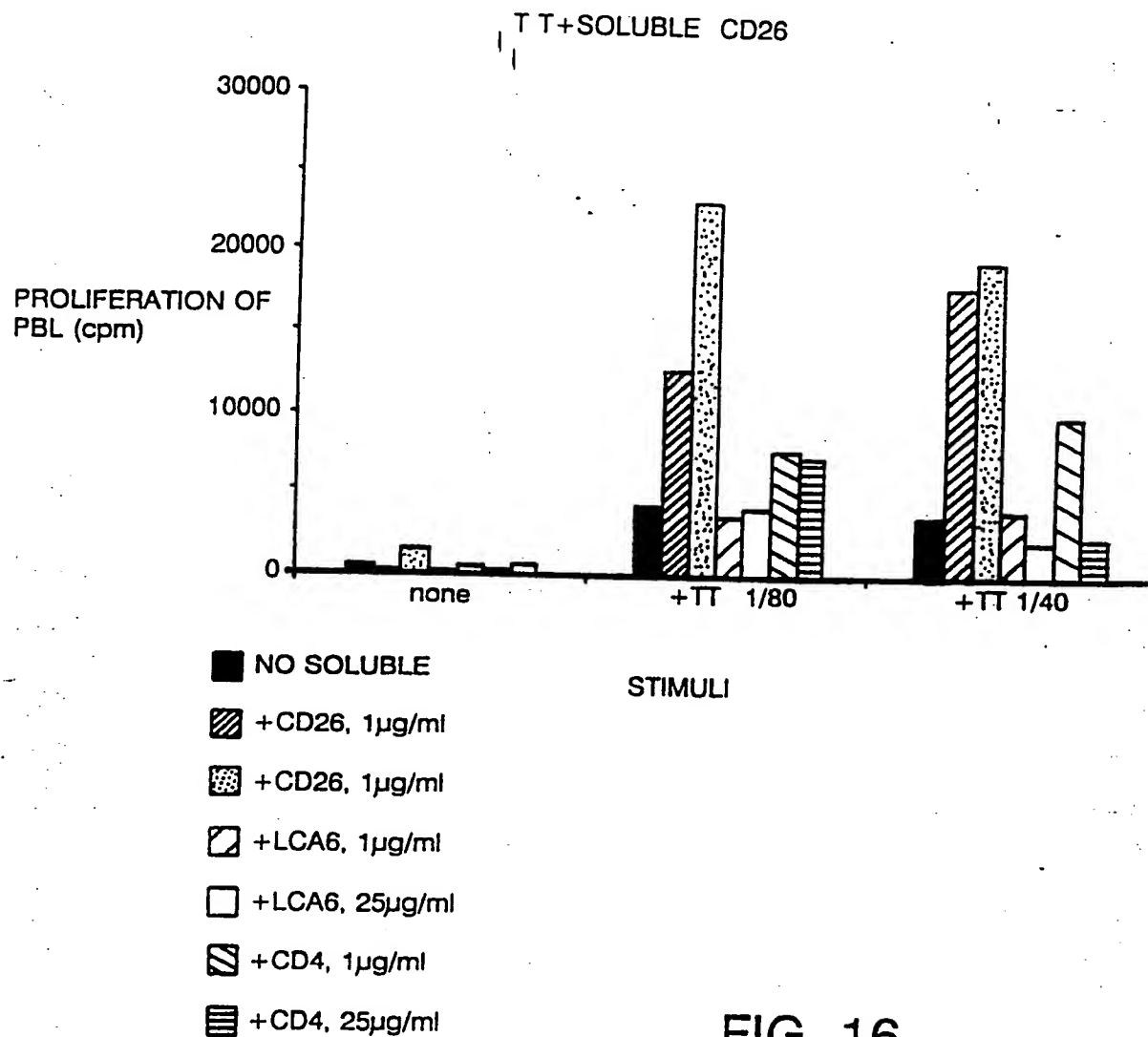
FIG. 15

FIG. 16

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5)   :C07K 3/28, 7/06, 15/28; C12N 15/00, 15/02, 15/11; G01N 33/50
US CL   :

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EMBL Data Library, issued 16 July 1991, Y. Misumi et al., "Primary structure of human liver dipeptidyl peptidase IV", accession number HSPCHDP7, entire document.	1, 3, 6, 10, 17, 19, 20-25, 30-37
Y	Journal of Biological Chemistry, Volume 263, Number 32, issued 15 November 1988, W. Hong et al, "Membrane orientation of rat gp110 as studied _in vitro_ translation, pages 16892-16898, see Fig. 1.	2, 4, 5, 9, 11-13
Y	Journal of Immunology, Volume 147, Number 8, issued 15 October 1991, Y. Torimoto et al, "Coassociation of CD26 (dipeptidyl peptidase IV) with CD45 on the surface of human T lymphocytes", Abstract	14-17, 26, 28
Y	E. Harlow et al, "Antibodies: A Laboratory Manual" published 1988 by Cold Spring Harbor Laboratory (N.Y.), see pages 148-151, 158-159, 207-219, entire document.	16, 24, 25
Y	Genomics, Volume 10, issued 1991, J. L. Fernandez-Luna et al, "Characterization and expression of the human leukocyte-common antigen (CD45) gene contained in yeast artificial chromosomes", pages 756-763, especially pages 760-761.	18-23

| X | Further documents are listed in the continuation of Box C. | | See patent family annex. |

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion f the international search

08 OCTOBER 1992

Date of mailing f the international search report

27 OCT 1992

Name and mailing address f the ISA/
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No.   NOT APPLICABLE

Authorized officer

DAVID SCHREIBER

Telephone No.   (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

CAS, APS, MEDLINE, BIOSIS
search terms: CD26, CD45, clone, immunoprecipitation, coprecipitation, interaction, cell surface, dipeptidylpeptidase
IV, antigens, activation antigen, peptide fragments, p43, polypeptide, plasmid

BOX I. OBSERVATIONS WHERE CLAIMS WERE FOUND UNSEARCHABLE
2. Where no meaningful search could be carried out, specifically:

These claims read on the polypeptide fragments of CD26 represented by SEQ ID Numbers: 2 and 3, said polypeptide
being soluble under physiological conditions. Both of these polypeptides are hydrophobic and would not be soluble
under physiological conditions. It appears that applicants intended to claim the polypeptide fragments of CD26 minus
the polypeptide fragments represented by SEQ ID Numbers: 2 and 3.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/02892

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 27
because they relate to subject matter not required to be searched by this Authority, namely:

The claim reads on naturally occurring CD26.

2. [X] Claims Nos.: 7 and 8
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Please See Extra Sheet.

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any addtional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention frist mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest.
[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1))(July 1992)*

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	Scandinavian Journal of Immunology, Volume 34, Number 2, issued August 1991, M. T. Ferm et al, "Human MHC class 1 antigens are associated with a 90-kDa cell surface protein.", Abstract.	28-29
Y	Immunogenetics, Volume 25, issued 1987, C. LeGuern et al, "Sequence determination of a transcribed rabbit class II gene with homology to HLA-DQ$_\alpha$", page 104-109, see pages 106 and 107 and attached sequence alignment.	28-29